Joint morphology and cartilage composition in people following anterior cruciate ligament reconstruction

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The University of Melbourne
For my parents Wudao and Yuzhu

And all the people who have helped me
ABSTRACT

Anterior cruciate ligament reconstruction (ACLR) is the preferred treatment for ACL rupture; however, early onset of post-traumatic knee osteoarthritis (OA) is common in this patient group. Hence individuals with ACLR constitute an ideal model for elucidating the natural history and pathogenesis of post-traumatic OA due to the accelerated onset and progression of the disease.

Magnetic resonance imaging (MRI) techniques can provide valuable information about structural changes by quantifying joint morphology and cartilage composition. Recent studies in knee post-traumatic cohorts have reported MRI-derived findings including joint morphology (i.e., cartilage volume, cartilage defects, bone marrow lesions, BMLs) and cartilage composition (i.e., T2 values). These findings can be extended by comparing joint morphology of isolated ACLR patients versus patients with combined meniscal pathology and by investigating associations between baseline cartilage defects and longitudinal cartilage volume change.

Hence, the aim of this longitudinal study was to provide a greater understanding of the natural history and pathogenesis of post-traumatic OA by studying early structural changes identified on MRI in ACLR patients at 2.5 years post-surgery and at 2 years follow-up. Participants were categorised into three groups: (a) those with isolated ACLR, (b) those with combined ACLR and meniscal pathology, and (c) healthy controls. The current study was divided into three chapters, with first two chapters introducing joint morphology and one chapter introducing cartilage composition.

In Chapter 4, knee joint morphology was compared across the three participant groups at baseline. Both ACLR groups exhibited worse cartilage and subchondral bone features compared with healthy controls, and the combined ACLR group showed a higher prevalence of cartilage defects than the isolated ACLR group. Moreover, both ACLR
groups presented with cartilage defects at all cartilage sites, except for the medial tibia, while a high prevalence of BMLs was detected in the lateral tibia.

Chapter 5 reported joint morphology changes over the 2-year follow-up period. An increase in cartilage volume was found in both ACLR groups. This finding confirms the notion that cartilage swelling or hypertrophy is indicative of early cartilage degeneration. Cartilage defects remained unchanged in most ACLR participants; however, improvement of patellar cartilage defects occurred in the isolated group. This suggests that the natural course of cartilage defects is not a unidirectional process of progression, but that hypertrophic repair can occur in some regions. BMLs exhibited an improvement in the medial tibia in the combined ACLR group.

Chapter 5 also compared joint morphology changes over the 2 years between the three groups. The isolated ACLR group exhibited greater annual cartilage volume increase compared with both the control group and the combined ACLR group. Thus, the combined ACLR group exhibited a more advanced level of cartilage degeneration than the isolated ACLR group, given that cartilage swelling suggests early degeneration while cartilage loss suggests more advanced cartilage degeneration. Changes in cartilage defects did not differ between three groups. Improvement of BMLs was more prevalent at the lateral tibia in the isolated ACLR group compared with the control group, indicating BML resolution at this site over the follow-up time period.

Chapter 5 examined the association between baseline cartilage defects and BML scores and subsequent cartilage volume change over 2 years in all ACLR participants. Higher baseline cartilage defect scores were associated with greater cartilage volume increase in the lateral tibia and patella, while higher baseline BMLs score in the medial tibia were associated with cartilage volume loss. This indicates that pathologic changes in the cartilage and subchondral bone were associated with subsequent cartilage degeneration.
Chapter 6 reported the cartilage compositional T2 values in isolated ACLR and control participants. ACLR group exhibited higher T2 value in the medial femoral condyle at baseline than healthy controls, suggesting cartilage degeneration at this site. ACLR participants demonstrated decreased T2 value in the deep layer of lateral tibia from 2.5 to 4.5 years post-surgery, suggesting a partial restoration of cartilage composition.

In summary, the results of this thesis make a substantive contribution to the literature as they demonstrate the 2-year changes in joint morphology and cartilage composition in young individuals following ACLR. This thesis also provides evidence that the early features of post-traumatic OA appear to be associated with pathology in both the cartilage and subchondral bone, the nature of which depends upon the existence of concomitant meniscal pathology and the joint compartment.
Declaration

This is to clarify that:

i. This thesis comprises only my original work and has not been published elsewhere (except as indicated in the preface) or submitted for the award of another degree,

ii. Due acknowledgement has been made in the text to all other material used,

iii. The thesis is less than 100,000 words in length, inclusive of footnotes but exclusive of tables, references and appendices.
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Preface

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Student Researcher:

Xinyang Wang contributed to the conception of research questions and data analyses, performed the data analyses and prepared the first draft of the manuscripts in Chapters Four.

Principal Investigator:

Professor Adam Bryant, the study co-ordinator was responsible for management of all aspects of the study including recruitment of surgeons; employment and supervision of research assistant; budget management; overall timelines, and reporting to the funding body as well as being primary supervisor of the student researcher.

Additional Investigators:

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Wang, X. Knee structural changes from 2.5 to 4.5 years following anterior cruciate ligament reconstruction (ACLR) with and without combined meniscal injury. School of Health Sciences Graduate Student Research Colloquium, The University of Melbourne, October 2015.
## Abbreviation

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>ACL</td>
<td>Anterior cruciate ligament</td>
</tr>
<tr>
<td>ACLR</td>
<td>Anterior cruciate ligament reconstruction</td>
</tr>
<tr>
<td>ADAMTS-5</td>
<td>Adisintegrin and metalloproteinase with thrombospondin motifs 5</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>ANCOVA</td>
<td>Analysis of Covariance</td>
</tr>
<tr>
<td>AVN</td>
<td>Avascular Necrosis of bone</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BML</td>
<td>Bone Marrow Lesion</td>
</tr>
<tr>
<td>BTB</td>
<td>Bone-Patellar Tendon-Bone</td>
</tr>
<tr>
<td>CHESM</td>
<td>Centre for Health, Exercise and Sports Medicine</td>
</tr>
<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>CL</td>
<td>Close Loop</td>
</tr>
<tr>
<td>CV</td>
<td>Coefficient of Variation</td>
</tr>
<tr>
<td>dGEMRIC</td>
<td>delayed gadolinium-enhanced MRI of cartilage</td>
</tr>
<tr>
<td>HERC</td>
<td>Human Research Ethics Committee</td>
</tr>
<tr>
<td>HS</td>
<td>Hamstring graft</td>
</tr>
<tr>
<td>ICC</td>
<td>Intraclass Correlation Coefficient</td>
</tr>
<tr>
<td>ICRS</td>
<td>International Cartilage Repair Society</td>
</tr>
<tr>
<td>IQR</td>
<td>Interquartile range</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>KL</td>
<td>Kellgren and Lawrence</td>
</tr>
<tr>
<td>KOOS</td>
<td>Knee Osteoarthritis Outcome Survey</td>
</tr>
<tr>
<td>MOAKS</td>
<td>MRI Osteoarthritis Knee Score</td>
</tr>
<tr>
<td>MMP</td>
<td>Matrix Metalloproteinase</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>OA</td>
<td>Osteoarthritis</td>
</tr>
<tr>
<td>OR</td>
<td>Odds Ratio</td>
</tr>
<tr>
<td>PD</td>
<td>Proton Density image</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of Interest</td>
</tr>
<tr>
<td>STIR</td>
<td>Short Tau Inversion Recovery image</td>
</tr>
<tr>
<td>T2 values</td>
<td>T2 relaxation time</td>
</tr>
<tr>
<td>TNF</td>
<td>Tumor Necrosis Factor</td>
</tr>
<tr>
<td>WORMS</td>
<td>Whole Organ Magnetic Resonance Imaging Score</td>
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1.1 Thesis overview

Damage to the anterior cruciate ligament (ACL) is one of the most common knee injuries leading to early-onset knee osteoarthritis (OA). Furthermore, ACL-injured patients remain at an increased risk of developing premature knee OA even after ACL reconstruction (ACLR). The main aim of this thesis is to provide a greater understanding of the natural history and pathogenesis of post-traumatic OA by detecting early changes in joint morphology and cartilage composition in young people following ACLR.

To achieve the aim, OA-related joint morphological features including cartilage volume, cartilage defects and bone marrow lesions (BMLs) were examined using magnetic resonance imaging (MRI) at 2.5 years following ACLR and again 2 years later. These features were compared between: (i) patients with isolated ACLR, (ii) patients with combined ACLR and meniscal pathology, and (iii) healthy control participants. The association between baseline cartilage defects and BMLs score and subsequent cartilage volume change were examined in order to explore the effect of cartilage and subchondral bone pathology. Cartilage compositional status was investigated using MRI biomarker T2 mapping in patients with isolated ACLR at baseline and follow-up.

Chapter one is an overview of the thesis including a brief introduction to explain why ACLR patients are a population worthy of investigation.

Chapter two provides a review of literature related to the studies included in this thesis. Specifically, literature pertaining to MRI assessment in an OA population was summarised, and results of previous studies investigating OA-related MRI features in ACLR cohorts were synthesised, critically appraised and discussed. The findings of the
literature review were used to inform the cross-sectional and longitudinal observational studies in this thesis.

**Chapter three** introduces the common methodology of the studies contained in this thesis, including participant recruitment, descriptive information, MRI measurement and test-retest reliability of the MRI measurement.

**Chapter four** compares joint morphological features at baseline (i.e., 2.5 years following ACLR) between: (i) patients with isolated ACLR, (ii) patients with combined ACLR and meniscal pathology, and (iii) healthy control participants.

**Chapter five** describes the change of joint morphology from 2.5 to 4.5 years following ACLR in each group, and compares morphological change between the three groups. Furthermore, the association between baseline cartilage defects and BML scores and subsequent cartilage volume change is examined in the ACLR individuals.

**Chapter six** reports the cartilage compositional status using MRI biomarker T2 values in patients with isolated ACLR at baseline compared with healthy controls. The longitudinal change of cartilage T2 value is examined in ACLR individuals over 2-year follow-up.

**Chapter seven** summarises the main findings of this thesis, discusses the strengths and limitations of the research, presents a synopsis of the clinical implications, and suggests areas for future research.

1.2 **Introduction**

1.2.1 **Prevalence and impact of knee OA**

Osteoarthritis (OA) is a leading cause of joint pain, dysfunction and disability (Glyn-Jones et al., 2015). OA is known as a disease involving all structures of the joint, including articular cartilage destruction, subchondral bone remodeling,
osteophytes formation, ligamentous laxity, synovial inflammation and weakening of muscles (Hutton, 1989; Loeser et al., 2012). With respect to OA prevalence, the knee is the most commonly affected lower limb joint, and about 34% of women and 24% of men aged 60 years and older have knee OA (Pereira et al., 2011). The lifetime risk of developing knee OA is 44% over the age of 45 in black and white women, with higher risk among those who are obese or have a previous injury (Murphy et al., 2008). Although a range of conservative and surgical treatments are available, there is currently no cure for knee OA and the only established treatment for end-stage OA is costly joint replacement (Bennell et al., 2011).

Due to an absence of effective management, OA causes considerable burden to the individual and the society. Individuals with knee OA suffer from pain, loss of function, reduced quality of life and depression, and in 2010, OA was estimated to cause 17.1 million years lived with disability (YLD) globally (Murray et al., 2012; Vos et al., 2012). From a societal perspective, OA affects 1.8 million people in Australia (AIHW, 2015). In 2012, OA-related health-care expenditure was about $3.75 billion. Taken the loss of productivity and wellbeing into consideration, the overall cost of OA was estimated to be $22 billion per annum (Arthritis Australia, 2014).

1.2.2 Post-traumatic phenotype of knee OA and clinical relevance

Osteoarthritis is considered an end-stage disease representing a common final consequence caused by different risk factors (Bennell et al., 2012; Chu et al., 2015; Cicuttini et al., 2014). For many years, aging was considered to be the strongest risk factor for knee OA (Sharma et al., 2006). However, OA is not only an age-related disease, but also a dynamic change of the joint closely associated with the response to insult or injury (Arden et al., 2006). Evidence shows that young people who suffer knee injuries have a 5-fold increased risk of subsequent knee OA after 30 years (Gelber et al., 2000). It is thus understood that OA is a heterogeneous condition, prompting the division of the
disease into different clinical phenotypes including post-traumatic, metabolic, ageing, genetic and pain (Bijlsma et al., 2011).

Post-traumatic OA, in contrast to idiopathic OA that occurs in people over 60 years old, predominately affects young and middle-aged adults. Typically diagnosed earlier in life and progressing much quicker than idiopathic OA, post-traumatic OA results in a longer period of pain and morbidity (Buckwalter et al., 2004).

At least 12% of knee OA is the phenotype of post-traumatic OA (Brown et al., 2006). However, a trend towards increasing sports participation and body mass index has led to an increased rate of joint injury in adolescents; thus, the prevalence and importance of post-traumatic OA is likely to continue increasing in the future (Parkkari et al., 2008). Considering young patients with knee injuries are at a significantly increased risk for knee OA 10 to 30 years later, they are often termed ‘young people with old knees’ (Gelber et al., 2000). Research in this patient group is therefore warranted.

1.2.3 Clinical importance of post-traumatic OA

Therapeutic dilemma for the young patient

Post-traumatic OA poses a therapeutic dilemma for clinicians since it disproportionately affects young people. Unfortunately, there is a treatment gap for young patients with knee OA. Various conservative approaches are typically always the first choice; however, the effectiveness of some conservative therapies is still uncertain (McAlindon et al., 2014; Zhang et al., 2010). Although exercise is potentially beneficial for early OA patients, the long-term effects and optimal dosage are yet to be determined (Kon et al., 2012). In fact, a large number of young individuals experience functional disability following knee injury, but their desire to continue high-level physical activity may further increase the likelihood of OA onset and progression (Noyes et al., 1983). Furthermore, young OA patients who wish to maintain high levels of physical activity are unlikely to be satisfied
with surgical knee replacement and restriction of activity. In addition, the durability of currently available knee implants is concerning given the high activity demands of young patients and the risk of implant revision in the long-term (Wen et al., 2014). It is not surprising that over 80% of orthopaedic surgeons surveyed agreed that better treatments are needed for young OA patients (Li et al., 2014). Therefore, management that is targeted to young post-traumatic OA patients is warranted.

Unique timing for early intervention

Unlike idiopathic OA, post-traumatic OA provides a unique opportunity for early intervention given that the injury event can be seen as a ‘start point’. Effective early management of post-traumatic OA would greatly benefit patients by minimizing tissue damage and reducing long-term morbidity (Anderson et al., 2011; Riordan et al., 2013). Also, early intervention will likely improve the efficiency of health service resources given that a large proportion of health care costs are presently directed towards later-stage management of OA (March et al., 2002; Sadosky et al., 2010). Thus, the traumatic event contributing to the initial ACL injury represents the disease starting point and, as such, an ideal indicator of when OA prevention strategies should be implemented.

1.3 Why is investigating ACLR patients important?

Whilst ACLR is frequently performed after ACL injury, the outcomes pertaining to OA-related change remain unclear

ACLR is performed in many patients although the rates vary from country to country. However, the efficacy of surgical management in the minimisation of OA-related change still needs to be investigated (Lohmander et al., 2007). It has been suggested that ACLR could reduce the risk of knee joint degeneration after 10 years; however, previous studies were based on radiographic assessment which is not sensitive to early joint changes
(Ajuied et al., 2014). Thus, it is necessary to detect joint morphology and cartilage compositional status in ACLR patients using more sensitive MRI techniques.

The ACLR knee represents the ideal research model for elucidating pathogenesis of OA

The degree of joint injury determines the speed of joint degeneration and healing potential (Anderson et al., 2011). In this respect, the ACLR knee is highly suitable for research given the accelerated onset and progression of post-traumatic OA. Whilst ACL injury typically causes only relatively mild damage to the articulating surfaces, the high-energy impacts often leads to an intra-articular fracture that extends through the cartilage and subchondral bone (Buckwalter et al., 2004). Accordingly, knee OA usually develops within the 10-15 years post-ACLR, while it occurs after only 2-5 years in those with severe intra-articular fractures (Anderson et al., 2011; Lohmander et al., 2007).

Understanding the natural history and pathogenesis of post-traumatic OA from animal models

For many years, animal models of OA have been used, with OA frequently induced via ACL transection. These ACL-transected OA models, closely resembling their human counterpart with ACL tear, are powerful research models for studying the natural history and pathogenesis of post-traumatic OA (Altman et al., 1990; Buckwalter et al., 2013). Although animal models are the cornerstone of preclinical research, our understanding of the natural history and pathogenesis of post-traumatic OA is still limited (Little et al., 2013). Investigating joint morphology and cartilage compositional status in a human ACLR cohort could provide further understanding of the natural history and pathogenesis of post-traumatic OA. This could contribute toward developing potential treatments to slow the onset and progression of knee OA.
Chapter 2: Literature review

2.1  ACL injury, ACLR and post-traumatic OA

2.1.1  ACL injury and ACLR

The ACL is the stabilizer of anterior-posterior displacement of the tibia on the femur, and it is one of the most commonly injured knee ligaments in young physically active individuals (Beynnon, 2005). Injury to the ACL is more likely to occur in young athletes compared with the general population and participation in vigorous sports has been found to increase the risk of ACL injury compared with participation in less intense sports (Arendt et al., 1995; Lohmander et al., 2007; Parkkari et al., 2008). The annual incidence of ACL rupture varies depending upon cohort characteristics and has been estimated at 50 to 81 per 100,000 persons in the general population and 152 to 3672 per 100,000 persons among professional athletes (Moses et al., 2012). ACL injuries are seen at high incidence in sports involving jump-landing, cutting and pivoting such as soccer, football, basketball and team handball (Granan et al., 2013).

Surgical reconstruction of ACL (ACLR) is a common surgical treatment and has been shown to improve stability and function of the ACL-deficient knee joint (Fu et al., 2000). The overall incidence of ACLR varies by countries, and the surgery is higher in Australia (52 per 100,000 person-year) than in other countries including Denmark (38), New Zealand (36.9), Norway (34), Sweden (32) and the United States (43.5) (Gianotti et al., 2009; Granan et al., 2008; Granan et al., 2009; Janssen et al., 2012; Lind et al., 2009; Mall et al., 2014) (Figure 2.1). Taking the data in the United States for example, it has been estimated that 75% patients were treated by ACLR among the 200,000 ACL ruptures annually, and the incidence of ACLR has doubled from 1994 to 2006, especially in female and patients younger than 20 years (Gottlob et al., 1999; Mall et al., 2014; Spindler et al., 2008).
2.1.2 Post-traumatic OA following ACLR and concomitant meniscal pathology

There is a misconception that ACLR can reduce the rate of knee OA (Marx et al., 2003), however cumulative literature has failed to identify better OA-related outcomes in ACLR patients compared to patients with ACL rupture treated non-operatively (Lohmander et al., 2007; Wen et al., 2014). Despite successful surgical procedure, ACLR seems have limited capacity to alter the outcomes in regard to OA development and progression (Lohmander et al., 2007). Given that there are a large amount of publications that interested in the incidence of OA after the ACLR, several systematic reviews and meta-analysis have been conducted to pool the data together. Using the criteria of radiographic OA of Kellgren and Lawrence (KL) grade 2 or higher, Lohmander et al. (2007) reported the incidence of radiographic OA at 10% to 90% after 10 to 20 years following the ACLR (Figure 2.2) (Lohmander et al., 2007). In the systematic review published later, Qiestad et al. (2009) reported an incidence of radiographic OA of 8% to 48% with a minimum of 10-year follow-up (Oiestad et al., 2009), while Claes et al. (2012) reported 28% in the same follow-up period (Claes, 2012). In another study, Chalmers et al. (2014) demonstrated an incidence of 35.3% in ACLR patients at 14 years after the
injury (Chalmers et al., 2014). Using a more strict criteria, Ajuied et al. (2013) demonstrated the incidence of moderate to severe OA at 20.3% by including the patients with KL grade 3 or 4 after 10 years (Ajuied et al., 2014). Putting together, the overall incidence of radiographic OA KL grade 2 is approximately 30% at 10 years following ACLR. The incidence of OA following ACLR is confounded by various factors, and potential predictors for higher OA incidence includes concomitant meniscal pathology, cartilage damage, patellar tendon graft, obesity, greater age at surgery, weak quadriceps and more than 6 months between injury and surgery (Keays et al., 2010; Kessler et al., 2008; Pinczewski et al., 2007; Seon et al., 2006).

![Figure 2.2 The incidence of radiographic OA after ACL injury and ACLR. Each data point represents a data set from 1 of 127 individual publications. Symbols: ● Nonsurgical treatment; ▼ primary suture or enhancement; ■ Reconstruction by autograft; ◊ Reconstruction by synthetic graft or allograft. (Lohmander et al., 2007)](image)

The meniscus is frequently damaged in combination with ACL injury at 40% to 60% (Noyes et al., 2012). The incidence of meniscal injuries were higher in certain sports such as basketball and team handball, and it could be the result of the impact forces to the knee at the moment of landing after jumping (Granan et al., 2013; Krosshaug et al., 2007). Given the fact that damaged meniscus is associated with adverse effects including higher joint contact stress and joint instability (Arno et al., 2013), it is not surprising to identify
that concomitant meniscal pathology is an important contributor to OA development after ACLR (Englund et al., 2012; Keays et al., 2010). In the systematic review including 33 studies, the author noted that nearly all studies found concomitant meniscal injury a significant risk factor for the OA development after ACL injury. Compared with knees with ACL injury alone, the prevalence of OA was 2-4 times higher in the knee with concomitant meniscal pathology (Oiestad et al., 2009).

2.1.3 Potential mechanism of post-traumatic OA following ACL injury

ACL injury triggers a remodelling process that encourages joint degeneration and both mechanical and biochemical factors play important roles in the development and progression of post-traumatic OA (Bijlsma et al., 2011; Kramer et al., 2011; Riordan et al., 2014).

Joint biomechanics and neuromuscular function are disrupted by the initial injury, and biomechanical factors contribute to OA development and progression (K. Bennell et al., 2012; Chu et al., 2015). In the acute phase, the ACL injury results in the damage to joint structures, hemorrhosis, and death of articular chondrocytes, leading to chondrocyte apoptosis and inflammatory response in the joint (Anderson et al., 2011; Riordan et al., 2014). While in the chronic phase, biomechanical alterations, such as joint instability, muscle weakness, inappropriate loading and impaired neuromuscular control, have been identified in ACL injured individuals (Ageberg, 2002; Andriacchi et al., 2006; Chaudhari et al., 2008).

Biochemical factors, mainly the inflammatory response, break the balance of synthesis and degradation of cartilage by activating proinflammatory cytokines and mediators (mainly Interleukin (IL)-1beta, tumour necrosis factor (TNF), IL-6, nitric oxide, matrix metalloproteinase (MMP) and a disintegrin and metalloproteinase with thrombospondin motifs 5 (ADAMTS-5))(Kramer et al., 2011; Manicone et al., 2008; Olson et al., 2014).
Those proinflammatory cytokines and mediators also involved in the metabolism of subchondral bone, causing damage to the structure (Kapoor et al., 2011).

The biomechanical and biochemical factors could interact thorough the mechanobiologic pathway. Mechanical alteration leads to changes via cellular signalling in cartilage metabolism (including CBP/p300-interacting transactivator with ED-rich tail 2 (CITED 2) and Wnt) and subchondral bone remodelling (including OPG/RANK/RANKL system, phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase (MAPK) and Wnt) (Castaneda et al., 2012; Yokota et al., 2011). Indeed, studies using animal models have observed that cartilage loading influences chondrocyte metabolism, proteoglycan synthesis, collagen fibril orientation and matrix metalloproteinase expression (Elder et al., 2001; Li et al., 2013; Sandy et al., 1984; Setton et al., 1999). In addition, the normal communication between cartilage and subchondral bone are exacerbated in the injured joint due to cartilage defects and vascular invasion, and the diffusion of small molecules causes the cartilage-subchondral bone unit degeneration (Burr et al., 2012; Lories et al., 2011; Pan et al., 2012). Finally, the cascade of events leads to the consequence of OA development(Figure 2.3).
Articular cartilage injury and subchondral bone damage are commonly found after the ACL injury, and the pathologic changes in cartilage and subchondral bone are important in the pathogenesis of post-traumatic OA (Kramer et al., 2011). The cartilage injury disrupts the metabolism of cartilage by increasing the synthesis of cartilage matrix, and it could cause cartilage apoptosis (Anderson et al., 2011; Buckwalter et al., 2004). Subchondral bone is also likely to sustain injury at the time of ACL injury in the form of bone marrow lesions (BMLs). BMLs are suggested to reduce the stress-dissipating capacity of the cartilage-subchondral bone unit, and this pathology could inhibit nutritional flow from the bone marrow to cartilage (Hunter, Zhang, et al., 2006). The effects of damage to the subchondral bone were also noted by histologically examining patient’s biopsy at 2 weeks following ACL injury (Johnson et al., 1998). Results demonstrated necrosis of osteocytes and empty lacuna in the involved subchondral bone at where post-traumatic BMLs were found (Johnson et al., 1998). Furthermore, joint injury could cause local avascular necrosis of bone (AVN), and ischaemia (decreased
blood flow) and increased intra-osseous pressure that could stimulate subchondral bone remodelling (Lafforgue, 2006; Welch et al., 1993). Increased subchondral bone remodelling is a necessary condition for the OA initiation, thus the ACL-injured patients are under the risk of OA development (Burr et al., 2012).

The mechanisms and molecular pathways that drive post-traumatic OA changes has been investigated from animal, cell and tissue culture studies (Anderson et al., 2011; Buckwalter et al., 2004). Importantly, cartilage injury and subchondral bone damage could be visualised on MRI in vivo. Detection of early joint changes prior to knee OA is necessary to understand the natural history and pathogenesis of the disease in humans, and identify risk factors that predispose to those changes in order to guide further intervention in ACLR populations.
2.2  Understanding OA from an imaging perspective

2.2.1  MRI is important to detect early joint changes

Until relatively recently, radiography was the most commonly used imaging modality for assessing joint structure in OA (Hunter & Felson, 2006). However, the features of cartilage and soft tissues are not directly observable on radiography, and joint space narrowing is a surrogate marker for cartilage loss (Guermazi et al., 2011). By contrast, MRI is capable of visualising the structural characteristics of both bony (i.e. subchondral bone) and soft tissues (i.e. cartilage and menisci). Therefore, MRI is considered as a preferable imaging modality for OA, as it is sensitive to subtle changes in knee joint structures without radiation (Eckstein et al., 2006; Guermazi et al., 2011).

MRI-derived measurement of joint morphology including cartilage volume, cartilage defects and BMLs provides valuable information about joint health (Hunter et al., 2011). Assessing the characterisation of cartilage morphology is central to OA, given that the loss of cartilage is the hallmark of the disease (Pelletier et al., 2013). Quantitative cartilage volume and semi-quantitative cartilage defects are two commonly used MRI-derived parameters that provide information about cartilage morphology (Eckstein et al., 2006). Apart from morphological assessment, cartilage defects could be seen as cartilage disruption due to the joint injury (Buckwalter et al., 2004). Subchondral bone also plays a critical role in initiation and progression of OA, and cellular signalling between subchondral bone and cartilage has been identified in joint degeneration (Burr et al., 2012; Lories et al., 2011; Pan et al., 2009). In particular, BMLs are notable features that are well-visualised using MRI (Roemer et al., 2009). BMLs are common in OA population and are identified as potent risk factors for joint degeneration (Felson et al., 2003).

Advances in MRI techniques have made it possible to evaluate cartilage composition such as proteoglycan loss and collagen disorganization, and these compositional MRI
techniques are referred to as ‘imaging biomarkers’ (Baum et al., 2013). Given that compositional changes occur prior to measureable changes in morphology, quantifying cartilage composition in at risk individuals presents the opportunity for detecting and managing early stage knee OA (Binks et al., 2013; Pedersen et al., 2011). Cartilage T2 mapping reflects the water content and collagen orientation in the tissue, and longer T2 values represent cartilage degeneration (Dunn et al., 2004; Liess et al., 2002; Lusse et al., 2000).

2.2.2 Cartilage volume

Cartilage volume is a quantitative assessment of cartilage morphology derived directly by delineating the surface of the cartilage plates from MRI images (Cicuttini et al., 1999). In fact, quantitative assessment of cartilage morphology involves a three dimensional reconstruction method, with cartilage volume and cartilage thickness the most frequently used parameters (Eckstein et al., 2006). Cartilage volume was suggested to be the first-step analysis compared with more comprehensive analysis such as sub-regional cartilage thickness (Eckstein et al., 2006). However, assessing longitudinal changes of cartilage volume is less technically challenging in comparison to cartilage thickness, given that subjective comparisons in cartilage thickness patterns at different time points is difficult (Eckstein et al., 2006). In addressing this technical issue, the cartilage thickness needs to be compared on a point-by-point basis by matching the bone interfaces from two or more MRI scans - a procedure that requires registration techniques (Eckstein et al., 2006; Wang et al., 2012). It requires higher technical expertise to measure cartilage thickness, while the measurement of cartilage volume has been validated in the previous studies (Burgkart et al., 2001; Cicuttini et al., 1999; Graichen et al., 2004). Furthermore, the advantage in measuring the subregional cartilage thickness could be compromised by the high standard variation compared with measuring cartilage volume in the whole compartment (Raynauld et al., 2008). Therefore, quantitative cartilage morphology will be measured as cartilage volume in the current study.
Cartilage volume has been used as the outcome parameter in OA research for many years, and it is suitable for large scale studies and multi-centre clinical trials (Eckstein et al., 2006; Hunter et al., 2015). In 117 radiographic OA patients, Cicuttini and colleagues (2004) reported the annual loss of cartilage volume as 7.0% in the medial tibiofemoral compartment and 7.8% in the lateral tibiofemoral compartment over 2 years (Cicuttini, Wluka, Wang, et al., 2004). In the patellofemoral joint, the annual rate of cartilage volume loss was 4.4% in the patella (Cicuttini et al., 2004). In another 2-year study, Raynauld and colleagues (2006) reported cartilage volume loss at 8.3% in the medial compartment and 3.5% in the lateral compartment among OA patients, and the presence of severe meniscus extrusion and meniscal tear are identified as the predictors for cartilage volume loss (Raynauld et al., 2006). OA patients with higher rate of cartilage volume loss have been associated with greater risk of knee replacement in the next 2 years (Cicuttini et al., 2004). The reproducibility was good to excellent for the measurement of cartilage volume, with the inter-observer reproducibility ranges from 0.4%-7.8% (coefficient of variation, CV) and the intra-observer reproducibility from 0.3%-6.4% (Wang et al., 2012). Therefore, the measurement of cartilage volume is accepted as a reliable method to understand the joint structure in OA research.

2.2.3 Cartilage defects

Cartilage defects are semi-quantitative measurement of cartilage morphology. Most cartilage-specific score systems were based on the arthroscopic evaluation called Outerbridge classification (Ding et al., 2005; Disler et al., 1995; Drape et al., 1998; Potter et al., 1998). The original arthroscopic Outerbridge classification evaluates the diameter of cartilage defects, while the modified MRI grading system observes the depth of the cartilage defects on a 0-4 scale (Ding et al., 2005; Outerbridge, 1961). The cartilage defects score are as follows: grade 0, normal cartilage; grade 1, focal blistering and intra-cartilaginous low-signal intensity area with an intact surface and base; grade 2, irregularities on the surface or base thickness < 50%; grade 3, deep ulceration with loss of thickness > 50%; grade 4, full-thickness cartilage wear with exposure of subchondral...
bone (Ding et al., 2005). The modified cartilage defects score system was exactly the same as the International Cartilage Repair Society (ICRS) classification (Brittberg et al., 2003). Moreover, as arthroscopic assessment of cartilage defects are frequently recorded using ICRS score, it will be consistent to use the same score for the MRI assessment. Thus, in the current thesis, cartilage defects will be evaluated using ICRS scoring system.

The clinical relevance of cartilage defects has been determined in a previous study, where cartilage defects were associated with subsequent cartilage volume loss in OA population and middle-aged adults (Ding et al., 2005; Roemer et al., 2012; Wluka et al., 2005). In a study with 117 symptomatic knee OA patients, participants with cartilage defects exhibited higher annual loss of cartilage volume (5.5%) compared with those without cartilage defects (3.2%) at the patellar over 2 years (Wluka et al., 2005). In another study including 177 participants with chronic knee pain, the prevalence of baseline cartilage defects was associated with increased risk of cartilage defects progression in both tibiofemoral and patellofemoral compartments over 6 months (Roemer et al., 2012). In 325 middle-aged (mean age 45 years) participants, baseline cartilage defect scores were associated with the annual cartilage volume loss over 2 years (Ding et al., 2005). Therefore, cartilage defects could be used as a semi-quantitative parameter assessing of cartilage morphology, and the presence of cartilage defects, reflecting the cartilage pathology, is associated with subsequent cartilage degenerative change in the OA patients and middle-aged adults.

### 2.2.4 Bone marrow lesions (BMLs)

A BML on MRI appears as an area of high signal intensity on proton density-weighted (PD) or T2-weighted or short tau inversion recovery (STIR) images (Braun et al., 2012). The term ‘bone marrow edema’ or ‘bone bruise’ was introduced in early 90s to describe the ill-defined hyper intensity in the bone marrow (Felson et al., 2003; Mink et al., 1989; Wilson et al., 1988). Histological examinations found the pathology of BMLs to consist of bone marrow necrosis, trabecular abnormalities, bone marrow fibrosis, and edema.
(Zanetti et al., 2000). In OA research, BMLs are associated with knee pain and function (Felson et al., 2001; Kim et al., 2013), cartilage loss (Hunter et al., 2006; Tanamas et al., 2010) and knee replacement in the short to medium term (Dore et al., 2010). There are several semi-quantitative MRI scoring systems for OA assessment that include assessment of BMLs such as the MRI Osteoarthritis Knee Score (MOAKS) and Whole Organ Magnetic Resonance Imaging Score (WORMS) (Hunter et al., 2011; Peterfy et al., 2004). Specifically, the MOAKS system was developed based on limitations identified in previous semi-quantitative assessments, and it is reliable and sensitive to investigate the natural history of OA and BML pathology (Guermazi et al., 2013; Hunter et al., 2011). Thus, the MOAKS system will be adopted in the current study.

The presence of BMLs was identified as a risk factor for subsequent cartilage loss in both OA patients and older community-dweller. In a study including 395 OA patients, BMLs size at baseline was associated with an increased risk of subsequent cartilage loss after 30 months follow-up, while the complete absence of baseline BMLs was related to a decreased risk of cartilage loss (Roemer et al., 2009). In support of these findings, a study with 107 knee OA patients found that baseline tibiofemoral BML volume was associated with an increase of full-thickness cartilage lesion area over 2 years (Driban et al., 2011). Among 177 patients with chronic knee pain, BMLs predicted the progression of cartilage defects over 6 months in the tibiofemoral joint (Roemer et al., 2012). In a study with 456 community-dweller (mean age 63 years), baseline BMLs were also associated with cartilage volume loss over 2.7 years in a dose–response manner, suggesting BMLs have a local effect on cartilage loss (Dore et al., 2010).

BMLs are not limited to OA. In fact, BMLs have been detected after trauma and injury (Baranyay et al., 2007; Palmer et al., 1997), and trauma-induced BMLs represent the footprint of the injury mechanism such as ACL injury (Sanders et al., 2000; Viskontas et al., 2008). It has been suggested that traumatic BMLs should be differentiated from non-traumatic BMLs, given that these BMLs are distinct in pathology, prognosis and
complications (Roemer et al., 2009; Roemer et al., 2014). Indeed, trauma-induced BMLs are associated with acute injury and subacute lesions as a result of altered loading, while non-traumatic BMLs present in OA, vascular necrosis and diseases involving substantial inflammation such as polyarthritis and osteomyelitis (Roemer et al., 2009). Thus, while the presence of BMLs seems to lead to cartilage loss in the OA population, the association is required to be further explored in the post-traumatic population.

2.2.5 T2 mapping

T2 mapping is frequently referred to as an ‘imaging biomarker’ due to its inherent strengths in evaluating cartilage status and exploring OA pathogenesis non-invasively (Baum et al., 2013; Mosher et al., 2011). T2 mapping evaluates cartilage composition including water content and orientation of the collagen by measuring T2 relaxation time value (referred to as the ‘T2 value’). In early studies, the T2 mapping technique was used in asymptomatic volunteers (Dardzinski et al., 1997; Mosher et al., 2000; Smith et al., 2001). Those studies suggested that the cartilage T2 values were proportional to the water content, and the change in T2 values was also associated with aging and spatial variation of the cartilage tissue.

T2 mapping is argued to be appropriate in the current PhD study compared with other compositional MRI sequences such as T1rho and delayed gadolinium enhanced cartilage MRI (dGEMRIC), given its availability on most MRI scanners, does not require contrast, and has acceptable scanning times (David-Vaudey et al., 2004; Glyn-Jones et al., 2015). Since cartilage compositional changes precede detectable morphological changes, T2 mapping is a useful non-invasive biomarker that evaluates cartilage degeneration in the early stage of OA non-invasively (Baum et al., 2013).

T2 mapping has been validated in OA-related studies. Higher T2 values were found in OA patients compared with healthy control individuals (Dunn et al., 2004; Mosher et al., 2011; Stahl et al., 2007), and increased T2 values were thought to represent cartilage degeneration (Nishioka et al., 2012; Nissi et al., 2006; Prasad et al., 2013). T2 mapping
has also been included as an outcome measure into the multi-centre study Osteoarthritis Initiative (OAI), which is sponsored by the National Institutes of Health (NIH) and includes over 4000 participants with 9 years follow-up (Peterfy et al., 2008). T2 technique was concluded as having good discriminatory power due to its relatively small reproducibility errors compared with changes in diseased cartilage (Baum et al., 2011).

In summary, MRI has been proven to be a sensitive and powerful tool for investigating the joint structural morphology and cartilage compositional status in OA research. The knowledge of subtype post-traumatic OA could be enhanced by investigating an ACLR population.
2.3 MRI findings following ACLR

2.3.1 Cartilage volume and cartilage thickness following ACLR

Given that there is a limited number of studies assessing quantitative cartilage morphology following ACLR, both cartilage volume and thickness assessment are included in this section. Table 2.1 provides participant characteristics of previous studies, and Table 2.2 provides a summary of the main findings of these studies.

Of the eight studies identified and included, four are longitudinal (Eckstein et al., 2015; Frobell, 2011; Frobell et al., 2009; Su et al., 2013) and four are cross-sectional in design (Andreisek et al., 2009; Li et al., 2013; Theologis et al., 2014; Van Ginckel et al., 2013). Sample sizes ranged from 15 to 92 with a mean participant age of 30.1 years. ACLR individuals included in these studies were predominantly male (69%), and body mass index (BMI) was approximately 24.1kg/m² in the three studies that reported this participant characteristic. The graft choice for ACLR was a mix of hamstring auto graft (semitendinosus and gracilis tendon, 39%), bone-patellar tendon-bone auto graft (39%) and allograft (22%). Seven studies reported the concomitant meniscal pathology with treatment consisting of meniscal repair, meniscal fixation or partial meniscectomy.

Three longitudinal studies were based on the same cohort (KANON trial) with 1-year, 2-year and 5-year follow-up post ACLR surgery (Frobell et al., 2009; Frobell et al., 2011; Eckstein et al., 2014). At 1-year and 2-year follow-up, cartilage volume/thickness increased in the medial femoral condyle and decreased in the trochlear (Frobell, 2011; Frobell et al., 2009). In the article published in 2015, the overall cartilage thickness was found increased in the tibiofemoral joint at 5-year post-surgery, particularly in the medial compartment (Eckstein et al., 2015). In another study including a 2-year follow-up, cartilage thickness increased in the lateral tibia (Su et al., 2013). These results suggested that, in contrast to cartilage loss commonly seen in the OA population, individuals with ACLR exhibited an increase in cartilage in the first several years following the surgery. Importantly, whilst previous studies have reported the change of cartilage volume and
thickness in the tibiofemoral joint, cartilage change in the patella has not been identified post-ACLR.

Four cross-sectional studies reported cartilage thickness in ACLR individuals compared with healthy control participants (Li et al., 2013; Van Ginckle et al., 2013) as well as contralateral uninjured knees (Andreisek et al., 2009; Theologis et al., 2014). Compared with healthy controls, ACLR participants exhibited no significant difference in any cartilage site at 6 months (Van Ginckle et al., 2013) and 2.4 years post-surgery (Li et al., 2013). Compared with contralateral uninjured knees, ACLR knees exhibited thinner cartilage in the lateral tibia and lateral femoral condyle at 1 year post-surgery (Theologis et al. 2014), and cartilage thinning became evident in the sub-regions of the tibiofemoral joint after 7 years (Andreisek et al., 2009).

Limitations of these studies include mixed cohorts such as concomitant meniscal injury and different grafts for ACLR – factors that may greatly affect the consistency of findings. Importantly, no previous study has compared quantitative cartilage morphology between patients with isolated ACLR and those with concomitant meniscal pathology. Another important limitation of the KANON trial was the absence of a healthy control group, as outlined by Eckstein et al. (2015).

Thus, a longitudinal study including patients having undergone ACLR using the same graft is required to further explore early post-surgical changes in cartilage morphology. It would be reasonable to categorise ACLR individuals into two groups, that is, with or without concomitant meniscal pathology. Moreover, inclusion of a healthy reference group will enable quantification of ACLR-related cartilage changes versus those occurring naturally over time.
Table 2.1 Participant characteristics in studies assessing quantitative cartilage volume or cartilage thickness following ACLR

<table>
<thead>
<tr>
<th>Study</th>
<th>ACLR Participants</th>
<th>Gender (Male/Female)</th>
<th>Mean age (years)</th>
<th>BMI (kg/m²)</th>
<th>ACLR graft</th>
<th>Meniscal pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frobell et al. (2009)</td>
<td>58 in total, 34</td>
<td>42/16</td>
<td>26.7</td>
<td>NR</td>
<td>19HS/15BTB</td>
<td>11 meniscectomy or meniscal fixation</td>
</tr>
<tr>
<td>Frobell et al. (2011)</td>
<td>61 in total, 45</td>
<td>45/16</td>
<td>26.0</td>
<td>NR</td>
<td>Surgeon’s preference</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACLR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Meniscectomy or meniscal fixation; exact number NR</td>
</tr>
<tr>
<td>Su et al. (2013)</td>
<td>15</td>
<td>7/8</td>
<td>35.1</td>
<td>23.3</td>
<td>8HS/7 allograft</td>
<td></td>
</tr>
<tr>
<td>Eckstein et al. (2014)</td>
<td>121 in total, 92</td>
<td>89/32</td>
<td>26.1</td>
<td>NR</td>
<td>NR</td>
<td>69 meniscectomy or meniscal fixation</td>
</tr>
<tr>
<td>Andreisek et al. (2009)</td>
<td>52</td>
<td>28/24</td>
<td>33.3</td>
<td>NR</td>
<td>3HS/49BTB</td>
<td>21 medial tear, 23 lateral tear</td>
</tr>
<tr>
<td>Li et al. (2013)</td>
<td>30</td>
<td>30/0</td>
<td>28.5</td>
<td>23.9</td>
<td>13HS/17 allograft</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACLR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>11 meniscectomy, 10 meniscal repair</td>
</tr>
<tr>
<td>Van Ginckel et al. (2013)</td>
<td>15</td>
<td>8/7</td>
<td>26.8</td>
<td>25.1</td>
<td>15HS</td>
<td>No meniscal pathology</td>
</tr>
<tr>
<td>Theologis et al. (2014)</td>
<td>18</td>
<td>8/10</td>
<td>38.3</td>
<td>NR</td>
<td>6HS/12 allograft</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ACLR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>10 meniscal tear</td>
</tr>
</tbody>
</table>

BMI = Body Mass Index. NR = not reported. HS = hamstring graft. BTB = bone-patellar tendon-bone graft.
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>FU (years)</th>
<th>Reference</th>
<th>Cartilage parameter</th>
<th>ROI</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frobell et al. (2009)</td>
<td>Longitudinal</td>
<td>1</td>
<td>Baseline</td>
<td>Volume/thickness</td>
<td>MT, LT, MFC, LFC, P and Tr</td>
<td>Increased cartilage volume and thickness in MFC; Decreased volume and thickness in Tr.</td>
</tr>
<tr>
<td>Frobell et al. (2011)</td>
<td>Longitudinal</td>
<td>2</td>
<td>Baseline</td>
<td>Thickness</td>
<td>MT, LT, MFC, LFC, P and Tr</td>
<td>Increased thickness in MFC; Decreased thickness in Tr.</td>
</tr>
<tr>
<td>Su et al. (2013)</td>
<td>Longitudinal</td>
<td>2</td>
<td>Baseline</td>
<td>Thickness</td>
<td>MT, LT, MFC, LFC and P</td>
<td>Increased thickness in LT at the 2-year follow-up.</td>
</tr>
<tr>
<td>Eckstein et al. (2014)</td>
<td>Longitudinal</td>
<td>5</td>
<td>Baseline</td>
<td>Thickness</td>
<td>MT, LT, MFC, LFC, Total</td>
<td>Increased cartilage thickness in TFJ over 5 years, particularly in the medial compartment (MT and MFC).</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>medial TFJ and lateral TFJ</td>
<td></td>
</tr>
<tr>
<td>Andreisek et al. (2009)</td>
<td>Cross-sectional; 7 years after ACLR;</td>
<td>-</td>
<td>Contralateral knee</td>
<td>Thickness</td>
<td>MT, LT, MFC and LFC</td>
<td>Thinner cartilage in sub regions of MT, LT, MFC and LFC.</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>FU (years)</td>
<td>Reference</td>
<td>Cartilage parameter</td>
<td>ROI</td>
<td>Findings</td>
</tr>
<tr>
<td>-----------------------</td>
<td>-----------------------------</td>
<td>------------</td>
<td>----------------------------------------</td>
<td>---------------------</td>
<td>----------------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>Li et al. (2013)</td>
<td>Cross-sectional; 2.4 years after ACLR</td>
<td>-</td>
<td>Healthy controls (n=15) Thickness MT, LT, MFC and LFC</td>
<td>ACLR = controls</td>
<td>MT, LT, MFC and LFC</td>
<td>ACLR = controls</td>
</tr>
<tr>
<td>Van Ginckel et al. (2013)</td>
<td>Cross-sectional; 0.5 year after ACLR</td>
<td>-</td>
<td>Healthy controls (n=15) Volume MT, LT, MFC and LFC</td>
<td>ACLR = controls</td>
<td>MT, LT, MFC and LFC</td>
<td>ACLR = controls</td>
</tr>
<tr>
<td>Theologis et al. (2014)</td>
<td>Cross-sectional; 1 year after ACLR</td>
<td>-</td>
<td>Contralateral knee Thickness MT, LT, MFC and LFC</td>
<td>Thinner cartilage in the LFC and LT of operated knee</td>
<td>MT, LT, MFC and LFC</td>
<td>Thinner cartilage in the LFC and LT of operated knee</td>
</tr>
</tbody>
</table>

FU = Follow-up. ROI = region of interest. MT = medial tibia; LT = lateral tibia; MFC = medial femoral condyle; LFC = lateral femoral condyle; P = patella and Tr = trochlear. TFJ = tibiofemoral joint.
2.3.2  Cartilage defects following ACLR

Table 2.3 provides details of participant characteristics of previous ACLR studies assessing cartilage defects. Table 2.4 provides a summary of the main findings of in the studies included in Table 2.3.

To date, eight studies have assessed cartilage defects in ACLR participants. Six of these studies are longitudinal (Costa-Paz et al., 2001; Faber et al., 1999; Hanypsiak et al., 2008; Lee et al., 2012; Potter et al., 2012; Weninger et al., 2008), whilst the remaining two are cross-sectional in design (Culvenor et al., 2015; Michalitsis et al., 2015). Sample sizes ranged from 12 to 111 with a mean age of 31.1 years. 308 (74%) ACLR patients were male, and the average BMI was approximately 24.9kg/m² but was only reported in two studies. Graft choice for ACLR included hamstring auto graft (81%), bone-patellar tendon-bone auto graft (18%) and other grafts (1%). Six studies reported concomitant meniscal pathology, and for which treatment included meniscectomy and meniscal repair.

Six longitudinal studies reported the natural change of cartilage defects changes from 2 to 12 years following ACLR (Faber et al., 1999; Costa-Paz et al., 2001; Weninger et al., 2008; Hanypsiak et al., 2008; Potter et al., 2012; Lee et al., 2012). Of these, five studies reported a progression of cartilage defects in 24% to 100% of ACLR participants over a period of 2-11 years (Faber et al., 1999; Costa-Paz et al., 2001; Weninger et al., 2008; Potter et al., 2012; Lee et al., 2012). Only one study (Lee et al., 2012) reported an improvement in cartilage defects amongst 5% of participants in lateral femoral condyle and medial femoral condyle at two years following ACLR. The findings of these longitudinal studies suggest that cartilage defects in ACLR knees progressively worsen; however, in a small number of ACLR patients, cartilage defects actually improve over time. Furthermore, the distribution of cartilage defects is site-specific, that is, cartilage defects are more prevalent in lateral femoral condyle and medial femoral condyle compared with other sites.
One cross-sectional study categorised ACLR patients into three groups according to the time from injury to surgery (Michalitsis et al., 2013). Prevalence of high-grade cartilage defects in patients who had reconstruction more than 12 months after the injury was 5.5 and 12.5 times higher compared with the other two groups who underwent ACLR within 3 months and between 3 to 12 months after the injury (Michalitsis et al., 2013). These findings suggest that the timing of ACLR after the ACL injury may play a considerable role in the magnitude of cartilage degeneration; thus, a homogeneous ACLR cohort with a similar time since surgery is required in future studies to observe the change of cartilage defects (i.e. within 6 months after injury). Another cross-sectional study concluded that 17% ACLR patients had cartilage defects or osteophyte in the patellofemoral joint at one year after ACLR (Culvenor et al., 2015). Interestingly, these patients were categorised as having MRI-defined patellofemoral OA; however, it could be argued that MRI-based classification may overestimate OA prevalence, given that previous studies demonstrated a radiographic OA rates at around 30% at 10 years post-ACLR (Ajuied et al., 2014; Chalmers et al., 2014; Claes, 2012; Lohmander et al., 2007; Oiestad et al., 2009).

Given that cartilage defects were present in up to 74% of the patients with delayed ACLR (i.e. > 12 months) after the injury (Michalitis et al., 2013), the natural history of cartilage defects needs to be investigated in a homogeneous ACLR cohort.
<table>
<thead>
<tr>
<th>Study</th>
<th>ACLR participants</th>
<th>Gender (Male/Female)</th>
<th>Mean age (years)</th>
<th>BMI (kg/m²)</th>
<th>ACLR graft</th>
<th>Meniscal pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faber et al. (1999)</td>
<td>23</td>
<td>18/5</td>
<td>30.0</td>
<td>NR</td>
<td>23HS</td>
<td>6 medial tear, 7 lateral tear</td>
</tr>
<tr>
<td>Costa-Paz et al. (2001)</td>
<td>21</td>
<td>15/6</td>
<td>31.0</td>
<td>NR</td>
<td>21BTB</td>
<td>NR</td>
</tr>
<tr>
<td>Weninger et al. (2008)</td>
<td>45</td>
<td>31/14</td>
<td>27.6</td>
<td>NR</td>
<td>38HS, 6BTB, 1 quadriceps tendon</td>
<td>19 medial tear, 12 lateral tear</td>
</tr>
<tr>
<td>Hanypsiak et al. (2008)</td>
<td>44</td>
<td>31/13</td>
<td>26.0</td>
<td>NR</td>
<td>Mix of HS and BTB</td>
<td>20 medial tear, 30 lateral tear</td>
</tr>
<tr>
<td>Potter et al. (2012)</td>
<td>28</td>
<td>16/24</td>
<td>35.1</td>
<td>NR</td>
<td>5HS, 20BTB, 3 allograft</td>
<td>No meniscal pathology</td>
</tr>
<tr>
<td>Lee et al. (2012)</td>
<td>36</td>
<td>30/6</td>
<td>34.5</td>
<td>NR</td>
<td>36HS</td>
<td>15 meniscal injury</td>
</tr>
<tr>
<td>Michalitsis et al. (2013)</td>
<td>109</td>
<td>96/13</td>
<td>26.4</td>
<td>25.6</td>
<td>Mix of HS and BTB</td>
<td>69 meniscal tear</td>
</tr>
<tr>
<td>Culvenor et al. (2015)</td>
<td>111</td>
<td>71/40</td>
<td>30.0</td>
<td>26.0</td>
<td>111HS</td>
<td>32 medial tear, 28 lateral tear</td>
</tr>
</tbody>
</table>

BMI = Body Mass Index. NR = not reported. HS = hamstring autograft. BTB = bone-patellar tendon-bone autograft.
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>FU (years)</th>
<th>Reference</th>
<th>Score system</th>
<th>ROI</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faber et al. (1999)</td>
<td>Longitudinal</td>
<td>6</td>
<td>Baseline</td>
<td>Subjective thickness</td>
<td>LT and LFC</td>
<td>13 (57%) patients showed cartilage thinning in LFC</td>
</tr>
<tr>
<td>Costa-Paz et al. (2001)</td>
<td>Longitudinal</td>
<td>2.8</td>
<td>Baseline</td>
<td>Subjective 3-level grading system</td>
<td>MT, LT, MFC and LFC</td>
<td>5 (24%) patients with severe defects showed cartilage thinning in LFC.</td>
</tr>
<tr>
<td>Weninger et al. (2008)</td>
<td>Longitudinal</td>
<td>2</td>
<td>Baseline</td>
<td>Modified Outerbridge</td>
<td>T and F</td>
<td>31 (69%) patients showed cartilage defects progression at follow-up; no specific site.</td>
</tr>
<tr>
<td>Hanypsiak et al. (2008)</td>
<td>Longitudinal</td>
<td>12</td>
<td>Baseline</td>
<td>Presence/absence</td>
<td>MT, LT, MFC and LFC</td>
<td>At baseline, cartilage defects were 25 (46%) in LFC, 11 (20%) in MFC, 7 (13%) in LT and 3 (6%) MT. At follow-up, cartilage defects were 26 (59%) in LFC, 18 (41%) in MFC, 6 (13%) in LT and 15 (34%) MT.</td>
</tr>
<tr>
<td>Potter et al. (2012)</td>
<td>Longitudinal</td>
<td>11</td>
<td>Baseline</td>
<td>Modified Outerbridge</td>
<td>MT, LT, MFC, LFC, P and Tr</td>
<td>The risk of cartilage defects progressively increased over 11 years. Compared to baseline, the risk at year 7-11 were 50 times in LFC, 30 times in P and 19 times in MFC.</td>
</tr>
</tbody>
</table>
Lee et al. (2012) | Longitudinal | 2 | Baseline | WORMS | MT, LT, MFC, LFC and P | 10 (27%) patients showed cartilage defects progression, 2 (5%) patients showed improvement. LFC, MFC and P showed more changes than other site.

Michalitsis et al. (2013) | Cross-sectional | - | Three groups based on the time from injury to surgery: (A) 0-3 months; (B) 3-12 months; and (C) over 12 months | ICRS | MT, LT, MFC, LFC, P and Tr | The prevalence of cartilage defects: group C > group A and B. The prevalence of cartilage defects were A 11 (34%), B 19 (38%) and C 26 (74%). Most cartilage defects were found in MFC and LFC.

Culvenor et al. (2015) | Cross-sectional; 1 year after ACLR | - | Partial- or full-thickness cartilage defects | Modified Outerbridge score: grade 0 = normal cartilage; grade 1 = chondral softening or blistering; grade 2 = shallow superficial ulceration, fibrillation or fissuring < 50% of depth; grade 3 = deep ulceration, fibrillation, fissuring or a chondral flap > 50% of depth; grade 4 = full-thickness chondral wear with exposure of subchondral bone (Potter et al. 1998). ICRS = International Cartilage Repair Society: grade 0 = normal cartilage; grade 1 = superficial lesions, fissures and cracks; grade 2 = fraying, cartilage defects < 50% of depth; grade 3 = cartilage defects > 50% of depth; grade 4 = complete loss of cartilage thickness (Kleemann et al., 2005).
Given that post-traumatic BMLs are related to the initial ACL injury, studies assessing BMLs following ACL injury and ACLR will both be included in this section. Although the earlier papers described ‘bone bruise’ on MRI after knee injury, the findings were not specific targeted to the ACL injured population (Bretlau et al., 2002; Mink et al., 1989; Tung et al., 1993; Vellet et al., 1991). Therefore, previous studies investigating ACL cohort were included, with nine studies following ACL injury and seven studies post-ACLR. Table 2.5 provides details of participant characteristics of previous studies. Table 2.6 provides a summary of the main findings of the studies included in Table 2.5.

One longitudinal study followed 17 patients (10 male) for 2 months after the ACL injury, and increased BMLs volume were found within the first 2 weeks and decreasing after 1 month (Szkopek et al., 2012). Eight cross-sectional studies reported the BMLs 3-12 weeks after the ACL injury (Bisson et al., 2013; Graf et al., 1993; Kaplan et al., 1999; Kijowski et al., 2012; Nishimori et al., 2008; Spindler et al., 1993; Viskontas et al., 2008; Yoon et al., 2011). The number of participants in these cross-sectional studies ranged from 25 to 171. 282 male participants (52%) were at a mean age of 27.2 years, and body BMI were 24.7kg/m² in the studies that reported participant characteristics. Three studies reported the graft of ACLR, however all MRI scan was taken before the reconstruction (Nishimori et al., 2008; Yoon et al., 2011; Kijowski et al., 2012). All the studies mentioned above reported concomitant meniscal pathology.

These studies following ACL injury affirmed that post-traumatic BMLs are pronounced in the first 3-6 weeks - as a ‘footprint’ of the injury. Lateral compartment presented the most BMLs after the injury due to the pivot-shit mechanism, in which the lateral tibia subluxated anteriorly with impaction of the posterior lateral tibia on the anterior femur (Spindler et al., 1993). BMLs also occur in themedial compartment as the result of contrecoup injury at compensatory varus knee alignment, with internal rotation of the
femur and anterior subluxation of the tibia (Kaplan et al., 1999; Yoon et al., 2011). Furthermore, the high loads imposed on the knee at the time of ACL injury not only results in damage to the subchondral bone, but also to cartilage and meniscal tissue. Indeed, three studies (Nishimori et al., 2008, Yoon et al., 2011, Bisson et al., 2013) found that the presence of BMLs in the lateral compartment was associated with a higher prevalence of cartilage defects and meniscal tears. Similarly, Kijowski et al. (2012) found a significant positive association between the presence of a cortical depression fracture and meniscal tear after the ACL injury. These findings suggest that higher-energy ACL injury that causes bone fracture could also lead to concomitant BMLs and meniscal tears. Taken these results together, the presence and size of BMLs should be examined relative to other joint structural abnormalities such as cartilage defects.

Seven studies have assessed longitudinal change in BMLs from 1 to 12 years following ACLR (Costa-Paz et al., 2001; Faber et al., 1999; Frobell, 2011; Frobell et al., 2009; Hanypsiak et al., 2008; Stein et al., 1995; Theologis et al., 2011). The number of participants in these studies ranged from 9 to 61. On average, 166 (70%) participants were male. The graft choice for ACLR was reported in four studies, and there was a mix of hamstring auto graft (46%), bone-patellar tendon-bone auto graft (53%) and allograft (1%) (Stein et al., 1995; Faber et al., 1999; Costa-Paz et al., 2001; Frobell et al., 2009). Five studies reported concomitant meniscal pathology in ACLR participants.

These longitudinal studies reported a variable natural history for BMLs amongst ACLR patients and the temporal changes in BMLs were site-specific. Typically, post-traumatic BMLs are most frequently located in the lateral tibia and lateral femoral condyle whilst lateral compartment BMLs are present in 54%-100% of patients. For this reason, it is not surprisingly that several previous studies have primarily focused on the BMLs in the lateral compartment post-ACLR (Faber et al., 1999, Frobell et al., 2011). The majority of BMLs in the lateral compartment (50-94%) resolve between one to six years following ACLR, and one study with 12-year follow-up reported complete resolution of BMLs in
all 44 participants (Hanypsiak et al., 2008). The progression of BMLs and newly developed BMLs in the lateral compartment occurred in about 30% ACLR individuals at 2-3 years following surgery (Stein et al., 1995, Frobell et al., 2011). By contrast, BMLs in the medial compartment were reported in 0-33% of ACLR patients, and the resolution ranged widely from 0-100% given the small number of baseline BMLs at this site. Given that BMLs in the medial compartment also occur after ACL injury and the baseline size was associated with cartilage volume loss, the natural history of BMLs in the medial compartment requires further investigation (Hunter et al., 2006; Kijowski et al., 2012; Yoon et al., 2011).

To date, no study has compared BMLs prevalence and size at 2.5 years following ACLR between individuals with and without combined meniscal injury. Furthermore, the natural history of BMLs - particularly those in the medial compartment- requires further investigation post-ACLR cohort.
<table>
<thead>
<tr>
<th>Study</th>
<th>Participants</th>
<th>Gender (Male/Female)</th>
<th>Mean age (years)</th>
<th>BMI (kg/m²)</th>
<th>ACLR graft</th>
<th>Meniscal pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stein et al. (1995)</td>
<td>20 ACLR</td>
<td>10/10</td>
<td>24.0</td>
<td>NR</td>
<td>2HS, 15BTB, 1 allograft</td>
<td>8 medial tear, 15 lateral tear</td>
</tr>
<tr>
<td>Faber et al. (1999)</td>
<td>23 ACLR</td>
<td>18/5</td>
<td>30.0</td>
<td>NR</td>
<td>23HS</td>
<td>6 medial tear, 7 lateral tear</td>
</tr>
<tr>
<td>Costa-Paz et al. (2001)</td>
<td>21 ACLR</td>
<td>15/6</td>
<td>31.0</td>
<td>NR</td>
<td>21BTB</td>
<td>NR</td>
</tr>
<tr>
<td>Hanypsiak et al. (2008)</td>
<td>44 ACLR</td>
<td>31/13</td>
<td>26.0</td>
<td>NR</td>
<td>Mix of HS and BTB</td>
<td>20 medial meniscal tear, 30 lateral meniscal tear</td>
</tr>
<tr>
<td>Frobell et al. (2009)</td>
<td>34 ACLR; 24 ACL injury</td>
<td>42/16</td>
<td>26.7</td>
<td>NR</td>
<td>19HS, 15BTB</td>
<td>11 meniscectomy or meniscal fixation</td>
</tr>
<tr>
<td>Frobell et al. (2011)</td>
<td>45 ACLR; 16 ACL injury</td>
<td>45/16</td>
<td>26.0</td>
<td>NR</td>
<td>Surgeon’s preference</td>
<td>Meniscectomy or meniscal fixation; exact number NR</td>
</tr>
<tr>
<td>Theologis et al. (2011)</td>
<td>9 ACLR</td>
<td>5/4</td>
<td>35.4</td>
<td>NR</td>
<td>NR</td>
<td>No meniscal pathology</td>
</tr>
<tr>
<td>Szkopek et al. (2012)</td>
<td>17 ACL injury</td>
<td>10/7</td>
<td>28.0</td>
<td>24.4</td>
<td>NA</td>
<td>2 medial tear, 5 lateral tear</td>
</tr>
<tr>
<td>Graf et al. (1993)</td>
<td>98 ACL injury</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>NA</td>
<td>17 medial tear, 12 lateral tear</td>
</tr>
<tr>
<td>Spindler et al. (1993)</td>
<td>54 ACL injury</td>
<td>NR</td>
<td>24.5</td>
<td>24.2</td>
<td>NA</td>
<td>20 medial tear, 30 lateral tear</td>
</tr>
<tr>
<td>Kaplan et al. (1999)</td>
<td>25 ACL injury</td>
<td>20/5</td>
<td>28.0</td>
<td>NR</td>
<td>NA</td>
<td>16 medial tear, 9 lateral tear</td>
</tr>
<tr>
<td>Viskonats et al. (2008)</td>
<td>100 ACL injury</td>
<td>69/31</td>
<td>35.1</td>
<td>NR</td>
<td>NR</td>
<td>37 medial meniscal tear, 33 lateral meniscal tear</td>
</tr>
<tr>
<td>Nishimori et al. (2008)</td>
<td>39 ACL injury</td>
<td>25/14</td>
<td>22.8</td>
<td>NR</td>
<td>39HS</td>
<td>33 lateral meniscal tear</td>
</tr>
<tr>
<td>Study</td>
<td>Participants</td>
<td>Gender (Male/Female)</td>
<td>Mean age (years)</td>
<td>BMI (kg/m²)</td>
<td>ACLR graft</td>
<td>Meniscal pathology</td>
</tr>
<tr>
<td>----------------------</td>
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<td>------------</td>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>Yoon et al. (2011)</td>
<td>81 ACL injury</td>
<td>22/59</td>
<td>29.0</td>
<td>25.0</td>
<td>81HS</td>
<td>41 medial meniscal tear, 44 lateral meniscal tear</td>
</tr>
<tr>
<td>Kijowski et al. (2012)</td>
<td>114 ACL injury</td>
<td>57/57</td>
<td>26.1</td>
<td>NR</td>
<td>56HS, 58BTB</td>
<td>34 medial meniscal tears, 51 lateral meniscal tears</td>
</tr>
<tr>
<td>Bisson et al. (2013)</td>
<td>171 ACL injury</td>
<td>89/82</td>
<td>25.2</td>
<td>24.9</td>
<td>NR</td>
<td>59 medial meniscal tear, 65 lateral meniscal tear</td>
</tr>
</tbody>
</table>

BMI = Body Mass Index. NR = not reported. NA = not applicable. HS = hamstring autograft. BTB = bone-patellar tendon-bone autograft.
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>FU (years)</th>
<th>Reference</th>
<th>Score system</th>
<th>ROI</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stein et al. (1995)</td>
<td>Longitudinal</td>
<td>3.3</td>
<td>Baseline</td>
<td>Three types: 1) ill-defined; 2) linear; and 3) fracture</td>
<td>MT, LT, MFC, LFC and P</td>
<td>Baseline BMLs were predominantly found in the LT and LFC. The BMLs prevalence was 20 (100%) in LT, 13 (65%) in LFC, 3 (15%) in MT, 0 in MFC and 1 (5%) in P. At follow-up, BMLs resolved in 24 (65%) participants and progressed in 13 (35%) participants.</td>
</tr>
<tr>
<td>Faber et al. (1999)</td>
<td>Longitudinal</td>
<td>6</td>
<td>Baseline</td>
<td>Presence/absence</td>
<td>LT and LFC</td>
<td>At baseline, all patients showed BMLs at LT and LFC. At follow-up, 15 (65%) in LT and 8 (35%) in LFC resolved over 6 years.</td>
</tr>
<tr>
<td>Costa-Paz et al. (2001)</td>
<td>Longitudinal</td>
<td>2.8</td>
<td>Baseline</td>
<td>Three types: I) diffuse signal with change of medullary component, II) localised signal with contiguity to the subjacent articular surface and III) disruption or depression of the normal cortical surface</td>
<td>MT, LT, MFC and LFC</td>
<td>At baseline, the number of type I, type II and type III BMLs were 13 (62%), 11 (52%) and 5 (24%). At follow-up, type I and type II BMLs were resolved in 23 (96%) patients. Type III BMLs were persistent in all 5 (100%) patients.</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>FU (years)</td>
<td>Reference</td>
<td>Score system</td>
<td>ROI</td>
<td>Findings</td>
</tr>
<tr>
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</tr>
<tr>
<td>Hanypsiak et al. (2008)</td>
<td>Longitudinal</td>
<td>12</td>
<td>Baseline</td>
<td>Presence/absence</td>
<td>MT, LT, MFC and LFC</td>
<td>At baseline, BMLs were predominant in LT and LFC. The prevalence of BMLs was 37 (69%) in LFC, 29 (54%) in LT, 3 (6%) in MFC and 9 (17%) in MT. At follow-up, all patients showed BMLs resolution.</td>
</tr>
<tr>
<td>Frobell et al. (2009)</td>
<td>Longitudinal</td>
<td>1</td>
<td>Baseline</td>
<td>Quantitative BML volume</td>
<td>MT, LT, MFC, LFC, P and Tr</td>
<td>At baseline, all patients had BMLs. After 1 year, BMLs volume gradually decreased, and a complete resolution was found in 22 patients (38%). 17 patients (29%) showed increased BMLs volume. Author did not mention specific site of BMLs.</td>
</tr>
<tr>
<td>Frobell et al. (2011)</td>
<td>Longitudinal</td>
<td>2</td>
<td>Baseline</td>
<td>Quantitative BML volume</td>
<td>LT and LFC</td>
<td>At baseline, BMLs were 58 (61%) in LT and 47 (77%) in LFC. At follow-up, 54 (93%) BMLs in LT resolved and 44 (94%) in LFC resolved. 21 (34%) participants in LT and 15 (25%) participants in LFC had new BMLs.</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>FU (years)</td>
<td>Reference</td>
<td>Score system</td>
<td>ROI</td>
<td>Findings</td>
</tr>
<tr>
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<tr>
<td>Theologis et al. (2011)</td>
<td>Longitudinal</td>
<td>1</td>
<td>Baseline</td>
<td>Quantitative BML volume</td>
<td>MT, LT, MFC and LFC</td>
<td>At baseline, the presence of BMLs was 7 (78%) in LT, 5 (56%) in LFC, 2 (22%) in MFC and 3 (33%) in MT. At follow-up, nearly 50% of BMLs resolved.</td>
</tr>
<tr>
<td>Szkopek et al. (2012)</td>
<td>Longitudinal</td>
<td>0.2</td>
<td>Baseline</td>
<td>Estimation of BML volume; BML intensity (1-4 scale)</td>
<td>MT, LT, MFC and LFC</td>
<td>The BMLs volume was larger in the LFC compared with MFC. The BML volume increased within the first 2 weeks and began decreasing after 1 month. The BML intensity showed same changes as the BML volume.</td>
</tr>
<tr>
<td>Graf et al. (1993)</td>
<td>Cross-sectional; From first week to over 6 months following the injury</td>
<td>-</td>
<td>No reference</td>
<td>Descriptive information</td>
<td>MT, LT, MFC and LFC</td>
<td>47 (48%) patients with ACL injury had BML. All the BMLs were found within 6 weeks after the injury. The prevalence of BMLs was: 38 (81%) in LFC, 30 (64%) in LT, 12 (26%) in MT and 9 (19%) in MFC.</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>FU (years)</td>
<td>Reference</td>
<td>Score system</td>
<td>ROI</td>
<td>Findings</td>
</tr>
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</tr>
<tr>
<td>Spindler et al. (1993)</td>
<td>Cross-sectional; Within 3 month after the injury</td>
<td>-</td>
<td>No reference</td>
<td>Descriptive information</td>
<td>MT, LT, MFC and LFC</td>
<td>43 (80%) patients had BML. The prevalence of BMLs was: 37 (69%) in LFC, 29 (54%) in LT, 9 (17%) in MT and 3 (6%) in MFC. The high presence of BMLs in LT and LFC supports the mechanism of injury involves anterior subluxation of tibia with impaction of the posterior lateral tibia on the anterior femur.</td>
</tr>
<tr>
<td>Kaplan et al. (1999)</td>
<td>Cross-sectional; Within 4 weeks after the injury</td>
<td>-</td>
<td>No reference</td>
<td>Descriptive information</td>
<td>MT, LT, MFC and LFC</td>
<td>All patients (25) had BMLs in the medial tibia, and 24 (96%) had BMLs in lateral compartment. Whilst mechanism of BMLs in the lateral compartment is same as Spindler et al. (1993), BMLs in the medial compartment involves the mechanism of countercoup injury. It occurs at compensatory varus knee alignment, with internal rotation of the femur and anterior subluxation of the tibia. The impaction in the MFC is more posterior than in the LFC.</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>FU (years)</td>
<td>Reference</td>
<td>Score system</td>
<td>ROI</td>
<td>Findings</td>
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<tr>
<td>Viskonats et al. (2008)</td>
<td>Cross-sectional; Within 6 weeks after the injury</td>
<td>Different injury mechanisms (non-contact versus contact)</td>
<td>ICRS score</td>
<td>MT, LT, MFC and LFC</td>
<td>The prevalence of BMLs was higher in lateral compartment (85%) than in the medial compartment (40%). The prevalence of severe BMLs (grade 3 intensity) in the LT was higher in the non-contact group (81%) compared with in the contact group (36%).</td>
<td></td>
</tr>
<tr>
<td>Nishimori et al. (2008)</td>
<td>Cross-sectional; Within 6 weeks after the injury</td>
<td>Participants with concomitant lateral meniscal tear</td>
<td>Presence/absence</td>
<td>LT and LFC</td>
<td>The prevalence of BMLs was 29(73%) in LT, 34 (87%) in LFC. The presence of BMLs in LT and LFC was associated with lateral meniscal tear and cartilage defects.</td>
<td></td>
</tr>
<tr>
<td>Yoon et al. (2011)</td>
<td>Cross-sectional; Within 6 weeks after the injury</td>
<td>Participants with concomitant meniscal tear</td>
<td>Presence/absence</td>
<td>MT; LT; MFC and LFC</td>
<td>The prevalence of BMLs was 73% in LT, 68% in LFC, 24% in MFC and 26% in MT. ACLR individuals with concomitant meniscal injury had more BMLs compared with isolated ACLR patients in both medial and lateral side of the knee.</td>
<td></td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>FU (years)</td>
<td>Reference</td>
<td>Score system</td>
<td>ROI</td>
<td>Findings</td>
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</tr>
<tr>
<td>Kijowski et al. (2012)</td>
<td>Cross-sectional;</td>
<td>-</td>
<td>No reference</td>
<td>Presence/absence;</td>
<td>MT, LT, MFC, LFC, P and Tr</td>
<td>The prevalence of BMLs was 106 (93%) in LT, 88 (77%) in LFC, 57 (50%) in MT, 30 (26%) in MFC. The presence of cortical depression fracture was significant associated with meniscal tear.</td>
</tr>
<tr>
<td></td>
<td>Within 3 weeks after the injury</td>
<td></td>
<td></td>
<td>Quantitative BML volume</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bisson et al. (2013)</td>
<td>Cross-sectional;</td>
<td>-</td>
<td>No reference</td>
<td>Presence/absence;</td>
<td>MT, LT, MFC and LFC</td>
<td>The prevalence of BMLs was 145 (85%) in LT, 132 (77%) in LFC, 44 (26%) in MT and 11 (6%) in MFC. The presence of BMLs in LT and LFC was associated with lateral meniscal tear, while the presence of BMLs in MT and MFC were not associated medial meniscal tear.</td>
</tr>
<tr>
<td></td>
<td>Within 6 weeks after the injury</td>
<td></td>
<td></td>
<td>Percentage size of BMLs</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FU = Follow-up. ROI = region of interest. MT = medial tibia; LT = lateral tibia; MFC = medial femoral condyle; LFC = lateral femoral condyle; P = patella; Tr = trochlear. ICRS = International Cartilage Repair Society. Depth grade 1 = superficial; grade 2 = shallow; grade 3 = deep; grade 4 = extensive; grade 5 = generalised. Intensity grade 1 = mild, signal intensity of BMLs are lower than muscle; grade 2 = moderate, equal to muscle; grade 3 = severe, brighter than muscle. Szkopek et al. (2012) calculated the best approximation to the BML volume as ABC/3, where A was the widest distribution measured in the coronal plane, B was the deepest antero-posterior distribution in the sagittal plane and C was the highest cranio-caudal distribution in the sagittal plane.
2.3.4 Association between BMLs and cartilage morphology change

As mentioned, the presence of cartilage defects (section 2.2.3) and BMLs (section 2.3.4) have been associated with subsequent cartilage loss in OA population. These findings suggest that the initial ACL rupture-related injury to the articular cartilage and subchondral bone predispose cartilage to subsequent degeneration. However, the effect of cartilage defects and BMLs on cartilage volume is unclear and rarely reported following ACL injury/ACLR. Table 2.7 provides a summary of previous studies that reported the association between cartilage morphology change and BMLs.

The association between cartilage morphology change and BMLs have been examined in two longitudinal studies. Frobell et al. (2011) reported both changes in cartilage thickness and volume of BMLs in the lateral compartment during the first 2 years following ACLR, however no significant association was identified between newly developed BMLs between these two parameters (Frobell, 2011). Potter et al. (2012) used cartilage defects as the cartilage morphology outcome, and baseline BML size was associated with cartilage degeneration (i.e. increased cartilage defects score) in the first 3 years at lateral tibia and in the first 2 years at lateral femoral condyle (Potter et al., 2012). It is noteworthy that the association was based on the data in the lateral compartment, while the association is not clear in the medial compartment.

The effects of damage to the cartilage and subchondral bone were examined histologically in patients at 2 weeks following ACL injury by Johnson et al. (1998). Results demonstrated degenerative change in the cartilage overlying BMLs together with the necrosis of osteocytes and empty lacuna in the involved subchondral bone (Johnson et al., 1998). Clearly, the longitudinal effects of BMLs on cartilage integrity could not be inferred given the cross-sectional nature of the study, and in vivo MRI studies could reveal clinical relevant information.

Therefore, a longitudinal study examining the association between BMLs and cartilage morphology (i.e. defects and volume) is urgently needed with the exploration extending
to both the medial and lateral compartments. Furthermore, given that previous studies have reported the association in the first 2-3 years following surgery, subsequent studies of similar design are required to substantiate the earlier findings.
Table 2.7 Summary of the association between cartilage thickness, cartilage defects and BMLs following ACLR or ACL injury

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Score system</th>
<th>ROI</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frobell et al. (2011)</td>
<td>Longitudinal; 2 years after ACLR</td>
<td>Cartilage thickness; Quantitative BML volume</td>
<td>LT and LFC</td>
<td>Cartilage thickness changes were not related to BMLs volume in the lateral compartment over 2-year follow-up.</td>
</tr>
<tr>
<td>Potter et al. (2012)</td>
<td>Longitudinal; 3 years after ACLR</td>
<td>Cartilage defects (Modified Outerbridge score); BML size</td>
<td>LT and LFC</td>
<td>BML size Increased cartilage defects score was associated with in the first 3 years in LT and first 2 years in LFC.</td>
</tr>
</tbody>
</table>

ROI = region of interest. MT = medial tibia; LT = lateral tibia; MFC= medial femoral condyle; LFC = lateral femoral condyle. Modified Outerbridge score: grade 0 = normal cartilage; grade 1 = chondral softening or blistering; grade 2 = shallow superficial ulceration, fibrillation or fissuring < 50% of depth; grade 3 = deep ulceration, fibrillation, fissuring or a chondral flap > 50% of depth; grade 4 = full-thickness chondral wear with exposure of subchondral bone. BML was assessed as absent, mild (<1 cm²), or severe (>1 cm²) (Potter et al., 2012).
### 2.3.5 T2 mapping following ACLR

Table 2.8 provides details of participant characteristics of previous studies assessing T2 values following ACLR, and Table 2.9 provides a summary of main findings in those studies.

Three longitudinal studies (Li et al., 2011; Potter et al., 2012; Su et al., 2013) and four cross-sectional studies (Bae et al., 2015; Li et al., 2015; Li et al., 2013; Van Ginckel et al., 2013) were included into the analysis. The number of participants ranged from 10 to 62, and 137 (80%) participants were male. BMI was reported in five studies (approximate 24.7\(\text{kg/m}^2\)). The graft choice of ACLR was reported in six studies including hamstring auto graft (43%), bone-patellar tendon-bone auto graft (12%) and allograft (45%) (Li et al., 2015; Li et al., 2013; Li et al., 2011; Potter et al., 2012; Su et al., 2013). Five studies reported concomitant meniscal pathology in the ACLR participants (Bae et al., 2015; Li et al., 2015; Li et al., 2013; Li et al., 2011; Su et al., 2013).

Three longitudinal studies reported the change in cartilage T2 values from 1 to 11 years following ACLR. Su et al. (2013) reported higher T2 cartilage values in the lateral tibia in ACL injured individuals compared with healthy controls at one month after the injury (Su et al., 2013). Given that longer T2 values represent cartilage degeneration, the higher cartilage T2 values in the lateral tibia represented the ACL introduced cartilage degeneration in the acute phase just like post-traumatic BMLs. Second, the change of T2 values reflect the dynamic change of the cartilage composition, and the longitudinal change was site-specific which may partly contribute to the joint loading (Nishii et al., 2008; Souza et al., 2014). Specifically, T2 value exhibited a trend towards a decrease in the lateral tibia and an increase in the medial femoral condyle were observed over the first year (Li et al., 2011) and the first two years post-ACLR (Su et al., 2013). Decreasing T2 values in the lateral tibia suggests a partial recovery of cartilage composition in the first 2 years post-ACLR (Li et al., 2011; Sanders et al., 2000; Su et al., 2013). By contrast, the trend toward increasing T2 values in the medial femoral condyle indicates differential,
site-specific cartilage responses, that is, increased water content and deterioration of the collagen network (Baum et al., 2013). Whilst Potter et al. (2012) followed their ACLR cohort for 11 years, the authors only described that T2 values increased in the lateral compartment without reporting quantitative data (Potter et al., 2012).

Cross-sectional studies with control group comparisons have reported cartilage T2 values between 0.5-2.8 years after ACLR. Overall, these studies suggest cartilage degeneration (i.e. increased water content and disorganized collagen) in ACLR patients given comparatively higher T2 values. Specifically, Van Gincke et al. (2013) reported higher T2 values in the medial femoral condyle at six months post-surgery, whilst Li et al. (2013) found significantly higher T2 values in medial tibia, medial femoral condyle, lateral tibia, lateral femoral condyle and trochlea at two years following ACLR. Li et al. (2015) compared cartilage T2 values in patients with isolated ACLR, ACLR plus meniscal repair, and ACLR plus meniscectomy at 2.8 years post-surgery. Interestingly, ACLR patients with concomitant meniscal injury showed significantly higher cartilage T2 values compared with those with isolated ACLR, supporting the notion that concomitant meniscal injury increased the risk of cartilage degeneration (Li et al., 2015). A within-subject study by Bae et al. (2015) reported significantly higher medial femoral condyle T2 cartilage values in ACLR knees versus uninjured contralateral knees at three years post-surgery (Bae et al., 2015). However, it is important to note that bilateral comparisons post-ACLR is considered problematic given that compensatory biomechanical adaptations are transferred to the uninjured limb (Zabala et al., 2013).

Imaging biomarker T2 value at different cartilage regions in the medial femoral condyle and lateral tibia across an extended time-frame (i.e. over 2 years) following surgery is required to provide additional insight into ACLR-related cartilage compositional alterations.
<table>
<thead>
<tr>
<th>Study</th>
<th>ACLR participants</th>
<th>Gender (Male/Female)</th>
<th>Mean age (years)</th>
<th>BMI (kg/m²)</th>
<th>ACLR graft</th>
<th>Meniscal pathology</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li et al. (2011)</td>
<td>12</td>
<td>7/5</td>
<td>34.0</td>
<td>24.1</td>
<td>6HS, 6 allograft</td>
<td>6 medial tear, 6 lateral tear</td>
</tr>
<tr>
<td>Potter et al. (2012)</td>
<td>28</td>
<td>16/24</td>
<td>35.1</td>
<td>NR</td>
<td>5HS, 20BTB, 3 allograft</td>
<td>No meniscal pathology</td>
</tr>
<tr>
<td>Su et al. (2013)</td>
<td>15</td>
<td>7/8</td>
<td>35.1</td>
<td>23.3</td>
<td>8HS, 7 allograft</td>
<td>7 medial tear, 7 lateral tear</td>
</tr>
<tr>
<td>Van Ginckel et al. (2013)</td>
<td>15</td>
<td>8/7</td>
<td>26.8</td>
<td>25.1</td>
<td>15HS</td>
<td>No meniscal pathology</td>
</tr>
<tr>
<td>Li et al. (2013)</td>
<td>30</td>
<td>30/0</td>
<td>28.5</td>
<td>23.9</td>
<td>13HS, 17 allograft</td>
<td>10 meniscal repair, 11 meniscectomy</td>
</tr>
<tr>
<td>Li et al. (2015)</td>
<td>62</td>
<td>62/0</td>
<td>29.0</td>
<td>NR</td>
<td>22 HS, 40 allograft</td>
<td>21 medial tear, 23 medial meniscectomy</td>
</tr>
<tr>
<td>Bae et al. (2015)</td>
<td>10</td>
<td>7/3</td>
<td>33.8</td>
<td>27.4</td>
<td>NR</td>
<td>3 medial tear, 5 lateral tear</td>
</tr>
</tbody>
</table>

BMI = Body Mass Index. NR = not reported. HS = hamstring autograft. BTB = bone-patellar tendon-bone autograft.
<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>FU (years)</th>
<th>Reference</th>
<th>ROI</th>
<th>Laminar analysis</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Li et al. (2011)</td>
<td>Longitudinal</td>
<td>1</td>
<td>Baseline; Healthy controls (n=10)</td>
<td>MT, LT, MFC, LFC and P</td>
<td>Full-thickness; Superficial layer and deep layer</td>
<td>At 1 year following ACLR, T2 values showed a trend towards a decrease in the LT and an increase in MFC. ACLR &gt; controls at baseline and year 1. Laminar analysis: ACLR &gt; controls in the deep layer of LT at baseline.</td>
</tr>
<tr>
<td>Potter et al. (2012)</td>
<td>Longitudinal</td>
<td>11</td>
<td>Baseline</td>
<td>LT, LFC and P</td>
<td>Full-thickness</td>
<td>T2 value increased in the LFC and P compared with the values at year 1; no quantitative T2 data.</td>
</tr>
<tr>
<td>Su et al. (2013)</td>
<td>Longitudinal</td>
<td>2</td>
<td>Baseline; Healthy controls (n=16)</td>
<td>MT, LT, MFC, LFC and P</td>
<td>Full-thickness; Superficial layer and deep layer</td>
<td>ACLR decreased in LT over 2 years. ACLR also showed a trend towards increase in the MFC. ACLR &gt; controls in LT at baseline and year 1. ACLR &gt; controls in MFC at baseline and year 2. Laminar analysis: T2 values in the LT decreased at superficial layer and increased at deep layer from baseline to year 2.</td>
</tr>
<tr>
<td>Van Ginckel al. (2013)</td>
<td>Cross-sectional; 6 months after ACLR</td>
<td>-</td>
<td>Healthy controls (n=15)</td>
<td>MT, LT, MFC, LFC and P</td>
<td>Full-thickness</td>
<td>ACLR &gt; controls at the MFC</td>
</tr>
<tr>
<td>Study</td>
<td>Design</td>
<td>FU (years)</td>
<td>Reference</td>
<td>ROI</td>
<td>Laminar analysis</td>
<td>Findings</td>
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<tr>
<td>Li et al. (2013)</td>
<td>Cross-sectional</td>
<td>2.4 years after ACLR</td>
<td>Healthy controls (n=15)</td>
<td>MT, LT, MFC, LFC, P and Tr</td>
<td>Full-thickness</td>
<td>ACLR &gt; controls in MT, MFC, LT, LFC and Tr.</td>
</tr>
<tr>
<td>Li et al. (2015)</td>
<td>Cross-sectional</td>
<td>2.8 years after ACLR</td>
<td>Three groups: (A) Isolated ACLR; (B) ACLR + medial meniscal repair; and (C) ACLR + medial meniscectomy</td>
<td>MT, LT, MFC and LFC</td>
<td>Full-thickness</td>
<td>Cartilage T2 values in the medial compartment: ACLR + meniscal repair &gt; isolated ACLR in MFC; ACLR + meniscectomy &gt; isolated ACLR in MFC; There was no difference between group B and group C</td>
</tr>
<tr>
<td>Baeet al. (2015)</td>
<td>Cross-sectional</td>
<td>3 years after ACLR</td>
<td>Contralateral uninjured knees</td>
<td>MT, LT, MFC and LFC</td>
<td>Full-thickness; Superficial layer and deep layer</td>
<td>ACLR = controls at all sites. Laminar analysis: ACLR knees &gt; contralateral knees in the superficial layer of MFC.</td>
</tr>
</tbody>
</table>

FU = Follow-up. ROI = region of interest. MT = medial tibia; LT = lateral tibia; MFC = medial femoral condyle; LFC = lateral femoral condyle; P = patella and Tr = trochlear.
People who undergo ACLR are at an increased risk of developing post-traumatic knee OA after 10-15 years. MRI techniques provide valuable information regarding the natural history and pathogenesis of OA by quantifying joint morphology and cartilage composition in patients following ACLR. Although recent studies have reported MRI-derived findings including cartilage volume, cartilage defects, BMLs and cartilage T2 values, previous studies are conceptually limited for a number of reasons: (i) knee joint morphology is rarely compared between individuals with isolated ACLR and those with concomitant meniscal pathology; (ii) associations between baseline cartilage defects and BMLs and longitudinal change in cartilage volume has, unlike the older OA population, not been examined in this young post-traumatic cohort; (iii) cohorts included in previous studies were heterogeneous with respect to graft type (i.e., participants underwent ACLR using a variety of different auto- and allografts). A longitudinal study with a larger, more homogeneous cohort is required. Given that Frobell et al. (2011) reported information about knee joint morphology in the first two years following ACLR, the current study will target the individuals having undergone ACLR 2.5 year’s prior and these individuals will be followed for 2 years.

Hence, the main aim of this thesis is to provide a greater understanding of the natural history and pathogenesis of post-traumatic OA by detecting early changes in joint morphology and cartilage composition in people following ACLR. The specific aims of this thesis are as follows:

(i) To examine joint morphology at 2.5 years following ACLR between (a) patients with isolated ACLR, (b) patients with combined ACLR and meniscal pathology, and (c) healthy control participants (Chapter 4);
(ii) To investigate the change of joint morphology from 2.5 to 4.5 years following ACLR between (a) patients with isolated ACLR, (b) patients with combined ACLR and meniscal pathology, and (c) healthy control participants (Chapter 5);

(iii) To examine whether baseline cartilage defects and BMLs scores are associated with the magnitude of cartilage volume change over 2 years in the ACLR participants (Chapter 5);

(iv) To compare imaging biomarker cartilage T2 values between patients with isolated ACLR at 2.5 years post-surgery and healthy control participants, (Chapter 6);

(v) To examine the change of cartilage T2 values from 2.5 to 4.5 years following ACLR in patients with isolated ACLR (Chapter 6).
Chapter 3: Methods

3.1 Introduction

This was a two-year prospective cohort study investigating the cartilage status of patients following ACLR using standard morphological and compositional techniques of MRI. Participants in this study were from a larger cohort study ‘Young people with old knees’, which was supported by the National Health and Medical Research Council (NHMRC Project Grant #628850).

The research in this thesis comprised of one cross-sectional study (Chapter 4) and two longitudinal studies (Chapters 5 and 6). The data analysed in all studies were derived from the same cohort of 130 participants, although the number of participants in each study varied, as outlined in the methods section in each chapter. Figure 3.1 shows the study aim for each chapter. This chapter describes the general methodology involved in all three studies, while variations specific to each study will be described in the relevant chapters.
Aims:
To compare cartilage morphology at 2.5 years following ACLR between: (i) patients with isolated ACLR, (ii) patients with combined ACLR and meniscal pathology, and (iii) healthy controls participants.

Chapter 4

Aims:
1) To investigate joint morphology change from 2.5 to 4.5 years following ACLR between: (i) patients with isolated ACLR, (ii) patients with combined ACLR and meniscal pathology, and (iii) healthy controls participants.

2) To examine whether baseline cartilage defects and BMLs scores are associated with the magnitude of cartilage volume change over 2 years in the ACLR participants.

Chapter 5

Aims:
1): To compare cartilage T2 values between participants with isolated ACLR at 2.5 years post-surgery and healthy controls participants;

2): To examine the change in cartilage T2 values over 2 years in ACLR participants.

Chapter 6

Figure 3.1 Outline of studies in this thesis
3.2.1 Recruitment

Participants with ACLR were recruited from Epworth Hospital Richmond in Melbourne Australia and from Pindara Hospital, Pacific Private Hospital and John Flynn Hospital on the Gold Coast, Queensland, Australia. Research assistants from the local hospitals screened the patients who had undergone a previous ACLR according to the inclusion and exclusion criteria, and sent letters on behalf of the surgeons to the potential participants. These patients were called one week after to enquire about their willingness to take part in the study. After providing informed consent, their details and medical records were transferred to the university research team. Healthy control participants were recruited from associated universities through campus advertisements. Recruitment took place from October 2011 to November 2012, and follow-up completed February 2015. The University of Melbourne and Griffith University Human Research Ethics Committees approved this study and all participants provided written informed consent (ethics approval number: 0932864.3 and PES/36/10/HREC). Figure 3.2 illustrates the flow of participants for this study.
Figure 3.2 Flow chart of participants in the study
3.2.2 Inclusion and exclusion criteria

The inclusion criteria for the ACLR participants were as follows:

i. Aged between 18-40 years;

ii. Body mass index (BMI) < 34 kg/m$^2$ (due to the issue of inaccurate skin marker placement for collection of biomechanical data required as part of the larger study);

iii. ACLR performed after an acute ACL tear (within 6 months following injury to minimise secondary damage to the cartilage and meniscus);

iv. Semitendinosis and gracilis (hamstring) graft used in the ACLR (to maintain the homogeneity of the ACLR participants);

v. 2-3 years following ACLR (as early cartilage changes are likely to be identified during this short-to-medium time frame).

The inclusion criteria for control participants were:

i. Aged between 18-40 years;

ii. BMI < 34 kg/m$^2$.

The exclusion criteria for the ACLR participants were as follows:

i. International Cartilage Repair Society (ICRS) cartilage defects grade > 2 on arthroscopy at baseline;

ii Other musculoskeletal, cardiovascular or neurological conditions;

iii Previous ACL surgery or subsequent knee surgery on the involved leg;
iv. Contraindications to MRI, including pacemaker, presence of shrapnel, metal in the eye, or claustrophobia.

The exclusion criteria for the ACLR participants were as follows:

i. Prior knee surgery or injury;

ii. Known lower limb injury or abnormality;

iii. Contraindications to MRI.

3.2.3 Surgical and rehabilitation procedure

All ACLRs were performed by one of four experienced orthopaedic surgeons with over 10 year experience using the same arthroscopically assisted technique. Ipsilateral semitendinosus and gracilis tendons were accessed through a three to four cm incision over the pesanserinus. The tendons were doubled to create a quadrupled graft, and a Closed Loop (CL) Endobutton (Smith and Nephew Endoscopy, Massachusetts, USA) was used for femoral fixation. Tibial fixation was achieved with an interference screw. Tibial and femoral tunnels were drilled using a 4.5 mm gauge drill, and a notchplasty was performed as required to ensure that graft impingement did not occur. The indications for the meniscal repair or partial meniscectomy procedure were based on the appearances of a tear at the time of surgery. Partial meniscectomy was performed in participants with non-repairable meniscal injuries that were deemed to be potentially symptomatic. No chondral surgery was undertaken as all lesions were less than ICRS grade 3.

Patients were discharged from hospital on the first postoperative day and weight bearing was encouraged on an as-tolerated basis without the use of braces or splints. A rehabilitation protocol that emphasised rapid restoration of knee range of motion and quadriceps
(particularly vastusmedialis) function was prescribed. All patients were provided with guidelines regarding their subsequent progression, the rate of which was based on the presence or absence of pain and swelling. Patients resumed full weight bearing by two to three weeks postoperatively, and they were asked to commence riding a stationary bicycle by week four in order to strengthen their quadriceps and hamstring muscles. At three to four months, the majority of patients was running and then started to participate in sports-specific drills from four months. Most patients return to full sport at nine to twelve months.

3.3 Descriptive information

3.3.1 Anthropometrics

Mass was measured to the nearest 0.1 kg using a weighing scale after removing shoes and bulky clothing. Height was measured to the nearest 0.1 cm (shoes removed) using a portable stadiometer. Mass and height were used to calculate body mass index (BMI) (kg/m²).

3.3.2 Sport activity level

The sports activity rating scale from the Cincinnati knee rating system was used to assess the activity level of the participants by considering both the frequency of play and the type of sports (Noyes et al., 1989) (Appendix I). Higher scores indicate higher level of sports participation (0-100).

3.3.3 KOOS questionnaire

The Knee injury and Osteoarthritis Outcome Score (KOOS) (Appendix II) was developed with the purpose of evaluating both short-term and long-term consequences of knee injuries (Roos et al., 1998). As an extension of the Western Ontario and McMaster Universities Arthritis Index (WOMAC), KOOS contains 42 items including five subscales: pain, other symptoms, function in daily living (ADL), function in Sport and Recreation (Sport/Rec), and knee related Quality of life (QOL). All items were scored on a Likert scale from 0 (no problems) to 4 (extreme problems), and the five dimensions were calculated separately as the sum of the items included. Scores were transformed to a 0-100 scale, with 0 representing
extreme knee problems and 100 representing no knee problems. ACLR participants were asked to complete the KOOS questionnaire with reference to the operated knee, while controls were asked to consider their knees in general.

3.3.4 Knee laxity

Anterior knee laxity was measured using the instrumented KT-1000 knee arthrometer (Medmetrics Corp, California, USA). The KT-1000 is the most commonly used device for knee ligament testing by measuring the anterior-posterior displacement of the tibia relative to the femur, which provides the information of knee anterior-posterior laxity. Participants were tested in the supine position in 30 degrees of knee flexion, and their knees were subjected to the anterior force at 15lbs, 20lbs and 30lbs (Hanten et al., 1987). An experienced examiner assessed the laxity of reconstructed knees and randomly assigned knees of control participants.

3.4 MRI acquisition

3.4.1 Cartilage morphology

MRI scan of the study knee was performed using two whole body MRI units in Melbourne (3.0T, Siemens MagnetomVerio, Erlangen, Germany) and the Gold Coast (1.5T, GE Healthcare Signa, Wisconsin, USA). Knees were imaged using T1-weighted 3D gradient recall in the sagittal plane and proton density (PD)-weighted fat-saturated spin echo acquisition in the coronal plane. The MRI technical parameters used in Melbourne included: T1-weighted, flip angle 10 degrees; repetition time 12.5 ms; echo time 4.9 ms; field of view 16cm; slice thickness 1.5mm; 60 partitions; 512 × 512 matrix; acquisition time 6 min 58 sec; PD-weighted, flip angle 155°, repetition time 2640 msec, echo time 37 msec, slice thickness 3 mm, field of view 16 cm, pixel matrix 256 × 256, acquisition time 1 min 55 sec. At the Gold Coast, these included: flip angle 55 degrees; repetition time 44ms; echo time 12 ms; field of view 16cm; slice thickness 1.5mm; 256 × 256 matrix; acquisition time 11 min 56 sec;
PD-weighted, flip angle 155°, repetition time 4000 msec, echo time 50 msec, slice thickness 3 mm, field of view 16 cm, pixel matrix 256 × 256, acquisition time 5 min 26 sec.

3.4.2 T2 mapping

Quantitative T2 relaxation time mapping was performed using one 3T MRI unit in Melbourne (Siemens Magnetom Verio, Erlangen, Germany). A sagittal multi-echo spin echo sequence was used, implementing a slice thickness of 3 mm; echo time/repetition time of 13.8, 27.6, 41.4, 55.2, and 69.0/1200 milliseconds; Flip Angle 180 degree; field of view 330 mm; matrix of 256×256 pixels (in-plane resolution: 0.42×0.42); and acquisition time of 8 min 16 sec.

3.5 MRI measurements

3.5.1 Cartilage volume

Cartilage volume was measured in the medial tibia, lateral tibia and patella using a method described in previous studies (Cicuttini et al., 1999; Peterfy et al., 1994). T1-weighted images were transferred to the Osiris v4.19 software (University Hospital of Geneva, Geneva, Switzerland). Cartilage segmentation involved manually tracing the bone interface and the cartilaginous joint surface slice-by-slice (Figure 3.3). Each cartilage compartment consisted of 20-30 slices. Cartilage volume (mm³) was determined by summing all the slice areas, and multiplying by the slice thickness (1.5mm). Cartilage segmentation for each knee joint took 2 hours at baseline, and this process required 4 hours at follow-up assessment given that baseline and follow-up cartilage volume needed to be measured paired, simultaneously. A trained observer (XW) who was blinded to the participants’ characteristics performed all MRI measures. In a pilot study, the intra-rater reliability was assessed using the MRIs from 10 participants on two occasions (time 1 and 2) with a time interval of 7 days, and the intraclass correlation coefficients (ICCs) were 0.995-0.997 for the cartilage volume (Table 3.1). The inter-rater reliability was determined between the observer (XW) and an experienced radiologist, and the ICCs were 0.985-0.993 (Table 3.2). In the main study, independent
measures of cartilage volume were cross-checked in a blinded manner by two observers, based on a random selection of 20% of the participants.

The annual change in cartilage volume and the annual percentage change were calculated to examine the longitudinal change of cartilage volume. The annual change was calculated as follows: \( \frac{(\text{follow-up cartilage volume} - \text{baseline cartilage volume})}{\text{time period between MRI scans}} \). Thus a positive value indicates cartilage volume increase.

![Figure 3.3 The segmentation of cartilage in the tibia (A) and patella (B)](image-url)
Table 3.1 The intra-rater reliability of cartilage volume (mm³) measurement

<table>
<thead>
<tr>
<th>Cartilage site</th>
<th>Cartilage volume at time 1 (mm³)</th>
<th>Cartilage volume at time 2 (mm³)</th>
<th>P value</th>
<th>ICC value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial tibia</td>
<td>1539.1 (1128.9, 1949.3)</td>
<td>1509.1 (1100.2, 1918.0)</td>
<td>0.15</td>
<td>0.995</td>
</tr>
<tr>
<td>Lateral tibia</td>
<td>1907.1 (1482.2, 2332.0)</td>
<td>1913.5 (1494.9, 2332.1)</td>
<td>0.70</td>
<td>0.996</td>
</tr>
<tr>
<td>Patella</td>
<td>2241.6 (1639.9, 2843.3)</td>
<td>2219.3 (1645.4, 2793.2)</td>
<td>0.30</td>
<td>0.997</td>
</tr>
</tbody>
</table>

Data presented as mean (95% confidence interval). P value was calculated using paired t-test.

Table 3.2 The inter-rater reliability of cartilage volume (mm³) measurement

<table>
<thead>
<tr>
<th>Cartilage site</th>
<th>Cartilage volume by experienced examiner (mm³)</th>
<th>Cartilage volume by student XW (mm³)</th>
<th>P value</th>
<th>ICC value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial tibia</td>
<td>1444.2 (1196.3, 1692.0)</td>
<td>1463.3 (1211.1, 1715.5)</td>
<td>0.40</td>
<td>0.985</td>
</tr>
<tr>
<td>Lateral tibia</td>
<td>1923.3 (1577.3, 2269.2)</td>
<td>1969.2 (1626.9, 2311.4)</td>
<td>0.31</td>
<td>0.987</td>
</tr>
<tr>
<td>Patella</td>
<td>2455.5 (2034.6, 2876.4)</td>
<td>2536.3 (2105.9, 2966.8)</td>
<td>0.08</td>
<td>0.993</td>
</tr>
</tbody>
</table>

Data presented as mean (95% confidence interval). P value was calculated using independent t-test.

3.5.2 Bone size

The bone size was measured as a covariate for the cartilage volume adjustment. The baseline bone area of medial and lateral tibial plateau was measured from images that were reformatted in the axial plane by Osirixv4.1.2 software (Pixmeo, Geneva, Switzerland). The area of tibial
plateau bone was measured on two images: the first image that shows both tibial cartilage and subchondral bone, and the next distal image. An average of the two areas was calculated as the tibial plateau bone area (mm²). The coefficient of variation (CV) for the medial and lateral tibial plateau size was 2.2% - 2.4% (Jones et al., 2000). The ICCs were 0.98-0.99 for the bone size (Figure 3.4).

Figure 3.4 The measurement of bone area in tibia (A) and patella (B). The bone area of tibial plateau was measured from the axial image. The area of lateral tibia (ROI-1), medial tibia (ROI-2) and patella (ROI-3) are shown in the figure. ROI, region of interest.
3.5.3 Cartilage defects

Cartilage defects were graded in the medial tibial plateau, medial femoral condyle, lateral tibial plateau, lateral femoral condyle and patella using the T1-weighted image. One examiner with over 8-year experience assessed cartilage defects using the ICRS cartilage defect score as follows:

- Grade 0, normal cartilage;
- Grade 1, focal blistering and intra-cartilaginous low-signal intensity area with an intact surface and base;
- Grade 2, irregularities on the surface or base thickness < 50%;
- Grade 3, deep ulceration with loss of thickness > 50%;
- Grade 4, full-thickness cartilage wear with exposure of subchondral bone (Ding, Cicuttini, et al., 2005) (Figure 3.5).

A cartilage defect was defined as a score of $\geq 2$ at any site that was present in at least two consecutive slices. The intra- and inter-observer reliability of cartilage defects measures (expressed as ICCs) was 0.89-0.94 and 0.85-0.93, respectively. In the longitudinal study, cartilage defects was defined as ‘progression’ if the cartilage defect score increased, ‘regression’ if the cartilage defect score decreased or ‘stable’ if the cartilage defect score did not change (this included those who had no defect at baseline and did not develop a defect) over 2 years.
Figure 3.5 The assessment of cartilage defects in the lateral compartment (A) and medial compartment (B). A: grade 2 cartilage defect in the lateral femoral condyle (arrowhead) and grade 3 cartilage defects in the lateral tibia (arrow). B: grade 4 cartilage defect in the medial femoral condyle (arrow).

3.5.4 Bone marrow lesions (BMLs)

BMLs were examined on the PD-weighted fat-saturated images in the medial tibia, medial femoral condyle, lateral tibia and lateral femoral condyle. BMLs at the patella were not graded because MRI images were only available in the coronal plane. The size of BMLs was graded from 0 to 3 based on the extent of regional involvement in 10 subregions (the anterior, central, and posterior regions of medial and lateral tibia; and central and posterior regions of medial and lateral femur) as following:

- Grade 0 = none;
- Grade 1 = < 1/3 of the subregional volume;
- Grade 2 = 1/3 – 2/3 of the subregional volume;
- Grade 3 = > 2/3 of the subregional region) (Hunter, Guermazi, et al., 2011) (Figure 3.6).
The intra- and inter-observer reliability (expressed as weighted kappa-values) in the reading of the BMLs ranged from 0.50-1.00 and 0.67-1.00. The score of BMLs was defined as the maximum score among the subregions in the subchondral bone. In the longitudinal study, BMLs were defined as ‘progression’ if the BML score increased, ‘regression’ if the BML score decreased or ‘stable’ if the BML score did not change (this included those who had no BML at baseline and did not develop a BML) over 2 years.

Figure 3.6 The assessment of BMLs with no BML (A), grade 1 BML (B), grade 2 BML (C) and grade 3 BML (D). BMLs are indicated with the arrow.
3.5.5 Compositional T2 values

T2 relaxation time value (in ms), in short “T2 value”, is an advanced MRI technique evaluating cartilage the compositional component (e.g. water content and orientation of the collagen). T2 relation time “images” were constructed by the multi-echo spin echo sequence using vendor-supplied software (Siemens syngoMapIt, Erlangen, Germany). Five Echo Times (TE) were included in the scan (i.e. TE = 13.8, 27.6, 41.4, 55.2 and 69 ms). Each T2 relaxation time “pixel” in T2 mapping was computed by fitting an exponential logarithm of T2 signal intensity decay (Figure 3.7 and Figure 3.8). Cartilage was directly segmented on this calculated T2 image, by manually tracing the boundary in the medial tibia, medial femoral condyle, lateral tibia, lateral femoral condyle, trochlear and patella, using v4.1.2 software (Pixmeo, Geneva, Switzerland), at window level 40 and window width 100 (Figure 3.8F). Segmentations were then transferred to an in-house program developed in Matlab (Mathworks, Mass., USA). Segmentations were overlaid on the first echo images (TE = 13.8ms) as shown in Figure 3.9, with colour-mapped T2 relaxation times. Full-thickness and superficial / deep laminar T2 values were calculated for all segmentations in all slices, with the laminar separation into the superficial and deep layer based on the midpoints between proximal and distal cartilage boundaries (Figure 3.10). Full-thickness and laminar T2 values were then averaged from the middle five slices of cartilage in the tibiofemoral joint (medial tibia, medial femoral condyle, lateral tibia and lateral femoral condyle), three slices in the trochlear, and ten slices in the patella. Pixels with relaxation time values greater than 100 msec on T2 mapping were removed from the data to reduce artefacts, which can be introduced by the magic angle effect, or synovial fluid at cartilage boundaries; these may cause overestimation of cartilage T2 values (Mosher et al., 2001; Ho et al., 2014). A trained observed (XW) performed all T2 segmentation. The intra-rater reliability of T2 relaxation time averages was determined from 10 participants on two occasions with a time interval of 7 days. ICCs for medial tibia, medial femoral condyle, lateral tibia, lateral femoral condyle, trochlear and patella were 0.95-0.98 (Table 3.3).
Figure 3.7 MRI images Five echo times with 13.8ms (A), 27.6ms (B), 41.4ms (C), 55.2ms (D), 69ms (E) and calculated image of T2 relaxation times (F).

Figure 3.8 T2 mapping with colour mapping for alternative visualisation. The colour spectrum represents the T2 value ranging from 0 to 100ms. Lower values are represented in blue, while higher values are represented in red.
Figure 3.9 The segmentation of cartilage regions in the medial tibia (A), medial femoral condyle (B), lateral tibia (C), lateral femoral condyle (D), trochlear (E) and patella (F).

Figure 3.10 Laminar analysis of cartilage T2 relaxation time value in superficial layer (A) and deep layer (B) at the lateral tibia.
Table 3.3: The intra-reliability of cartilage T2 averages (ms) measurement

<table>
<thead>
<tr>
<th>Cartilage site</th>
<th>T2 value at time 1 (95% confidence interval)</th>
<th>T2 value at time 2 (95% confidence interval)</th>
<th>P value</th>
<th>ICC value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial tibia</td>
<td>31.9 (28.3, 35.4)</td>
<td>32.2 (29.1, 35.4)</td>
<td>0.48</td>
<td>0.95</td>
</tr>
<tr>
<td>Medial femoral condyle</td>
<td>43.8 (40.6, 47.1)</td>
<td>43.3 (39.9, 46.7)</td>
<td>0.12</td>
<td>0.98</td>
</tr>
<tr>
<td>Lateral tibia</td>
<td>29.7 (26.5, 32.9)</td>
<td>29.2 (25.8, 32.6)</td>
<td>0.27</td>
<td>0.96</td>
</tr>
<tr>
<td>Lateral femoral condyle</td>
<td>44.5 (42.2, 46.8)</td>
<td>44.3 (42.1, 46.5)</td>
<td>0.25</td>
<td>0.96</td>
</tr>
<tr>
<td>Trochlear</td>
<td>51.1 (46.9, 55.3)</td>
<td>50.8 (46.8, 54.8)</td>
<td>0.41</td>
<td>0.98</td>
</tr>
<tr>
<td>Patella</td>
<td>36.0 (33.3, 38.6)</td>
<td>35.4 (32.9, 38.0)</td>
<td>0.06</td>
<td>0.98</td>
</tr>
</tbody>
</table>

Data presented as mean (95% confidence interval). P value was calculated using paired t-test.
3.6 Sample size calculation

No published studies from which an effect size for cartilage volume differences between ACLR groups could be found. Hence sample size was based on achieving a moderate effect size (i.e. 0.4) with 80% power and an alpha level of 0.05. Using this approach, a minimum of 22 participants per group (66 in total) was required for the cross-sectional baseline study. With regard to the longitudinal change of cartilage volume, a sample size of 32 participants is able to detect an effect size of 0.5 with 80% power and an alpha level of 0.05.

3.7 Statistical analysis

The assumptions of statistical testing including normality and homogeneity of variance were examined by visually inspecting the data and by using Kolmogorov-Smirnov test. Means and standard deviations are presented for parametric variables with normal distribution (i.e. cartilage volume and T2 values), while nonparametric variables (i.e. scores of cartilage defects and BMLs) are shown by median and interquartile range.

In the cross-sectional studies, the differences between the three groups were examined using one-way ANOVA or Kruskal-Wallis test for parametric or nonparametric variables respectively (Chapter 4). The comparison between 2 groups was analysed using independent samples t-tests or Mann-Whitney U (Chapter 6). Cartilage volume was compared between groups using analysis of covariance (ANCOVA) after adjusting for confounders including age, gender, BMI and bone size, given that these covariates have been identified as determinants of cartilage morphology (Cicuttini et al., 1999; Jones et al., 2000). Cartilage T2 values were compared between groups using ANCOVA after adjusting for age, gender and BMI, since these confounders are related to cartilage composition (Liebl et al., 2014). Chi square and Fisher exact test were used to compare proportions of cartilage defects and BMLs deemed progression, regression or stable
between groups. In the event of a significant main effect, *post hoc* comparisons were conducted if necessary using the Fisher's least significant difference (LSD) test or Mann-Whitney U test. Logistic regression was used to examine the prevalence of cartilage defects after adjusting for the same confounders as cartilage volume.

In the longitudinal studies (Chapter 5), paired t-test and Wilcoxon signed-rank test were used to examine the change within each group. Annual change in cartilage volume was compared between the three groups by ANCOVA adjusting for age, gender, BMI, baseline cartilage defect and baseline bone size. Chi-squared test and Fisher exact test were used to explore group difference in change in cartilage defects and BMLs (progression and regression). Logistic regression was used to examine the risk of progression in cartilage defects and BMLs between ACLR with or without meniscal injury after adjusting for age, gender and BMI. Multiple linear regressions were used to assess the association between quantitative cartilage volume change and semi-quantitative predictors (i.e. cartilage defects and BMLs) with the ACLR groups amalgamated.

All statistical analyses were performed using SPSS version 22 (IBM, Chicago) with significance accepted at $p < 0.05$. A $p$ value less than 0.05 (two-tailed) or a 95% confidence interval (CI) not including the null point was regarded as statistically significant.
Chapter 4: Cartilage morphology at 2–3 years following anterior cruciate ligament reconstruction with or without concomitant meniscal pathology


As outlined in the Preface author contribution for this chapter are the following: XW: contributed to study conception and design, analysis and interpretation of data and prepared draft of manuscript, managed manuscript submission and subsequent reviews; YW: study conception and design, analysis and interpretation of data; KLB: study conception and design, analysis and interpretation of data and obtaining of funding; TVW: study conception and design, analysis and interpretation of data and obtaining of funding; FMC: study conception and design, analysis and interpretation of data and obtaining of funding; KF: acquisition of data; DJS: acquisition of data; AVG: analysis and interpretation of data; ARD: acquisition of data; NG: acquisition of data; CV: provision of patients; JAF: provision of patients; TW: provision of patients; DGL: study conception and design and obtaining of funding; ALB: study conception and design, analysis and interpretation of data and obtaining of funding. All authors provided feedback on draft of this manuscript and approved the final manuscript.

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Cartilage morphology at 2–3 years following anterior cruciate ligament reconstruction with or without concomitant meniscal pathology

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Abstract

Purpose To examine differences in cartilage morphology between young adults 2–3 years post-anterior cruciate ligament reconstruction (ACLR), with or without meniscal pathology, and control participants.

Methods Knee MRI was performed on 130 participants aged 18–40 years (62 with isolated ACLR, 38 with combined ACLR and meniscal pathology, and 30 healthy controls). Cartilage defects, cartilage volume and bone marrow lesions (BMLs) were assessed from MRI using validated methods.

Results Cartilage defects were more prevalent in the isolated ACLR (69 %) and combined group (84 %) than in controls (10 %, P < 0.001). Furthermore, the combined group showed higher prevalence of cartilage defects on medial femoral condyle (OR 4.7, 95 % CI 1.3–16.6) and patella (OR 7.8, 95 % CI 1.5–40.7) than the isolated ACLR group. Cartilage volume was lower in both ACLR groups

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Comparing with controls (medial tibia, lateral tibia and patella, $P < 0.05$), whilst prevalence of BMLs was higher on lateral tibia ($P < 0.001$), with no significant differences between the two ACLR groups for either measure.

Conclusions Cartilage morphology was worse in ACLR patients compared with healthy controls. ACLR patients with associated meniscal pathology have a higher prevalence of cartilage defects than ACLR patients without meniscal pathology. The findings suggest that concomitant meniscal pathology may lead to a greater risk of future OA than isolated ACLR.

Level of evidence III.

Keywords Anterior cruciate ligament reconstruction · Meniscal injury · Cartilage · Osteoarthritis · Magnetic resonance imaging

Introduction

Anterior cruciate ligament reconstruction (ACLR) is commonly performed following ACL rupture in an effort to restore knee joint kinematics and stability. Although ACLR reduces the risk of subsequent meniscal tears and is thought to reduce the development of post-traumatic osteoarthritis (OA) in the years following ACL injury, recent studies have failed to identify substantial OA prevention-related advantages of surgical reconstruction compared to conservative management [1, 29, 33]. This may be due to post-ACLR mechanical and biochemical factors including residual instability [38], altered gait biomechanics [17] and chronic joint inflammation [19]. Moreover, concomitant meniscal injury was the most frequently reported risk factor for OA development following ACLR [1, 23, 29, 32, 33]. Meniscal tears are not always repairable and may require meniscal resection that has adverse effects on the mechanics of the tibiofemoral joint (TFJ). These effects include increased joint contact stress [24] and impaired stability [2]—both factors that increase the likelihood and severity of OA in the years following ACLR.

The patellofemoral joint (PFJ) has received relatively little attention although it, like the TFJ, is also susceptible to OA following ACLR [6, 20] and meniscectomy [13]. The mean incidence of PFJ OA at 10–15 years following ACLR is almost 50% [6, 34]. Since it has been suggested that the cartilage in the TFJ and PFJ have different properties (e.g. water content) [44] and is exposed to different mechanical loading patterns [20], cartilage adaptive response and the resultant degenerative changes may be nonuniform across knee compartments [20, 34].

Magnetic resonance imaging (MRI) studies examining the progression of joint structural deterioration have increased our understanding of the pathogenesis of OA. Indeed, reliable MRI-derived measures of cartilage morphology, such as cartilage defects and cartilage thickness/volume, provide valuable information pertaining to cartilage status [11]. Studies involving ACLR patients have reported changes in cartilage morphology from 6 months to 11 years post-surgery in both the tibia and femur [12, 15, 16, 25, 27, 36, 41, 45, 46]. In the PFJ, however, most studies have failed to identify significant patellar changes despite the fact that cartilage thinning has been reported in the femoral trochlea [15, 16]. With respect to structural changes following meniscectomy, previous MRI studies have shown cartilage defects and cartilage volume loss in the TFJ [5, 31] and PFJ [47]. It is noteworthy that whilst the isolated effects of ACLR and meniscal pathology have been investigated in separate studies, their combined effect on cartilage morphology remains unclear. Previous studies using compositional MRI have demonstrated higher T1rho and T2 values, suggesting early degeneration of cartilage tissue composition, in patients with ACLR and combined meniscal pathology compared with patients with isolated ACLR [26, 28, 43]. However, there is a lack of evidence demonstrating differences in cartilage morphology between the two groups of ACLR patients. Furthermore, most of the previous MRI studies are limited by the absence of an uninjured control group as well as small numbers of ACLR patients.

Subchondral bone is also susceptible to damage at the time of ACL injury, and bone marrow lesions (BMLs) are frequently observed on MRI as a ‘footprint’ of the injury mechanism [35, 40]. BMLs are thought to be caused by direct impact between the femur and tibia or abnormal loading [4, 40], and they have been associated with joint structural deterioration [4, 22]. Post-traumatic BMLs are typically located in lateral compartment following ACL injury, and previous studies have reported the presence, resolution and reemergence of lateral BMLs in the first several years after ACLR [15, 16, 36]. Thus, BMLs exhibit a changeable natural history following ACL injury and subsequent surgery [18].

In the light of these considerations, the primary aim of the present study was to compare TFJ and PFJ cartilage morphology (i.e. cartilage defects and cartilage volume) between: (i) isolated ACLR patients without meniscal pathology, (ii) patients with combined ACLR and meniscal pathology, and (iii) healthy control participants. It was hypothesized that: $H_1$ both groups of ACLR patients would exhibit lower cartilage volume and greater prevalence of cartilage defects compared with the control participants; $H_2$ patients with combined ACLR and meniscal pathology would exhibit lower cartilage volume and greater prevalence of cartilage defects compared with those with isolated ACLR.
Materials and methods

Participants with ACLR were recruited from October 2011 to November 2012. Inclusion criteria: (i) aged 18–40 years (with the upper age limit to reduce the likelihood of pre-existing OA); (ii) ACLR performed for an acute ACL tear within 6 months of injury; (iii) ACLR performed using semitendinosus and gracilis (hamstring) autograft; and (iv) ACLR performed 2–3 years previously. Participants were excluded if they had: (i) International Cartilage Repair Society (ICRS) cartilage defects grade >2 noted at time of ACLR or osteophytes on arthroscopy or X-ray at baseline; (ii) other musculoskeletal, cardiovascular or neurological conditions; (iii) previous ACL surgery or subsequent knee surgery on the involved leg; (iv) body mass index (BMI) > 34 kg/m²; and (v) contraindications to MRI. Eligible participants without concomitant meniscal pathology at the time of ACLR were assigned to the isolated ACLR group, and those who had concomitant meniscal pathology (i.e. meniscal injury, meniscal repair and partial meniscectomy) were assigned to the combined ACLR and meniscal pathology group. Healthy participants were recruited from associated universities using the following inclusion criteria: (i) aged 18–40 years and (ii) BMI < 34 kg/m². The exclusion criteria for healthy controls were: (1) prior knee surgery, (2) known lower limb injury or abnormality, and (3) contraindications to MRI. The University of Melbourne and Griffith University Human Research Ethics Committees approved this study, and all participants provided written informed consent.

There were no published studies from which an effect size for TFJ and/or PFJ cartilage volume differences between ACLR groups could be found. Hence, sample size was based upon achieving a moderate effect size (i.e. 0.4) with 80 % power and an alpha level of 0.05. Using this approach, a minimum of 22 participants per group (66 in total) was required for this cross-sectional study.

Procedure

All ACLRs were performed by one of the four orthopaedic surgeons with over 10-year experience using the same arthroscopically assisted technique. Ipsilateral semitendinosus and gracilis tendons were harvested through a 3–4-cm incision over the pes anserinus. Both tendons were doubled to create a quadrupled graft, and a Closed Loop (CL) Endobutton (Smith and Nephew Endoscopy, Mansfield, Mass., USA) was used for femoral fixation. Tibial fixation was achieved with an interference screw. The indications for the meniscal repair or partial meniscectomy procedure were based on the appearances of the tear at the time of surgery. Partial meniscectomy was performed in participants with nonrepairable meniscal injuries that were deemed to be potentially symptomatic. No chondral surgery was undertaken as all lesions were less than ICRS grade 3.

Participants were discharged from hospital on the first post-operative day, and weight bearing was encouraged on an as-tolerated basis without the use of braces or splints. The rehabilitation protocol emphasized rapid restoration of knee range of motion and quadriceps (particularly vastus medialis) function. All patients were provided with guidelines regarding their subsequent progression, the rate of which was based on the presence or absence of pain and swelling. Patients were asked to commence riding a stationary bicycle by week 4 post-operatively in order to strengthen their quadriceps and hamstring muscles. At 3–4 months, patients were allowed to commence straight line, followed by the commencement of sports-specific drills from 4 months.

Anthropometric and clinical assessments

Height, weight and BMI

Weight was measured to the nearest 0.1 kg (shoes and bulky clothing removed) using electronic scales. Height was measured to the nearest 0.1 cm (shoes removed) using a stadiometer. Weight and height were used to calculate body mass index (BMI; kg/m²).

Knee symptoms and function

The Knee injury osteoarthritis outcome score (KOOS) [39] was used to assess pain, function, symptoms, activities of daily living, sport and recreation function, and knee-related quality of life with higher scores indicating better results (0–100). ACLR participants were asked to complete the KOOS questionnaire with reference to their operated knee, whilst controls were asked to consider their knees in general.

Knee laxity

Anterior knee laxity was measured using the instrumented KT1000™ or KT2000™ knee arthrometer (Medmetrics Corp, California, USA). An experienced examiner assessed the laxity of reconstructed knees and randomly assigned knee of control participants.

MRI assessment

MRI scan of the study knee was performed using two whole body MRI units in Melbourne (3.0T, Siemens Magnetom Verio, Erlangen, Germany) and the Gold Coast
(1.5T, GE Healthcare Signa, Wisconsin, USA). Knees were imaged using T1-weighted 3D gradient recall in the sagittal plane for cartilage morphology assessment and proton density (PD)-weighted fat-saturated spin-echo acquisition in the coronal plane for BMLs assessment. The MRI technical parameters used in Melbourne included: T1-weighted, flip angle 10°, repetition time 12.5 ms, echo time 4.9 ms, field of view 16 cm, slice thickness 1.5 mm, 60 partitions, 512 × 512 matrix, acquisition time 6 min 58 s; PD-weighted, flip angle 155°, repetition time 2640 ms, echo time 37 ms, slice thickness 3 mm, field of view 16 cm, pixel matrix 256 × 256, acquisition time 1 min 55 s. At the Gold Coast, these included: flip angle 55°, repetition time 44 ms, echo time 12 ms, field of view 16 cm, slice thickness 1.5 mm, 256 × 256 matrix, acquisition time 11 min 56 s; PD-weighted, flip angle 155°, repetition time 4000 ms, echo time 50 ms, slice thickness 3 mm, field of view 16 cm, pixel matrix 256 × 256, acquisition time 5 min 26 s.

MRI analysis

Cartilage defects

Cartilage defects were graded on the medial tibial plateau, medial femoral condyle, lateral tibial plateau, lateral femoral condyle and patella. One examiner with over 7-year experience evaluated cartilage defects using the assessment derived from ICRS score as follows: grade 0 normal cartilage; grade 1 focal blistering and intra-cartilaginous low-signal intensity area with an intact surface and base; grade 2 irregularities on the surface or base and loss of thickness <50 %; grade 3 deep ulceration, with >50 % loss of thickness; and grade 4 full-thickness cartilage wear with exposure of subchondral bone [9]. The prevalence cartilage defect was defined as a score of ≥2 at any site that was present in at least two consecutive slices. Intra- and inter-observer reliability (expressed as intra-class correlation coefficients, ICCs) was 0.89–0.94 and 0.85–0.93, respectively.

Bone marrow lesions

BMLs were examined on the PD-weighted fat-saturated images in the medial tibia, medial femoral condyle, lateral tibia and lateral femoral condyle. BMLs at the patella were not graded because PD-weighted images were not available in the sagittal plane. The size of BMLs was graded from 0 to 3 based on the extent of regional involvement in 10 subregions (the anterior, central and posterior regions of medial and lateral tibia; and central and posterior regions of medial and lateral femur) as following: grade 0 none; grade 1 <1/3 of the subregional volume; grade 2 1/3–2/3 of the subregional volume; and grade 3 ≥2/3 of the subregional region [21]. The intra- and inter-observer reliability of cartilage defects measures (expressed as weighted kappa values) in the reading of the BMLs ranged from 0.50–1.00 and 0.67–1.00. The score of BMLs was defined as the maximum score among the subregions in the subchondral bone.

Cartilage volume and bone size

Cartilage volume was measured on the medial tibia, lateral tibia and patella using previously described techniques [48]. Cartilage volume was determined by manually tracing the bone interface and the cartilaginous joint surface from MR images on a slice-by-slice basis using the software Osiris (University of Geneva, Switzerland). Tibial plateau bone size was measured from axial images, and patellar bone volume was measured using the same method as for cartilage volume [47, 48]. One trained examiner read the MR images independently, blinded to participant group, and the ICCs for inter-reliability were above 0.99. The ICCs for inter-reliability between the examiner and experienced radiologist were 0.98–0.99.

The University of Melbourne and Griffith University Human Research Ethics Committees approved this study (ethics approval number: 0932864.3 and PES/36/10/HREC).

Statistical analysis

The groups were compared using one-way ANOVA or Kruskal–Wallis test for parametric or nonparametric variables, respectively. Fisher exact test was used to compare proportions in cartilage defects and BMLs. In the event of a significant main effect, post hoc comparisons were conducted using the Fisher’s least significant difference (LSD) test or Mann–Whitney U employing Bonferroni adjusted P value. Logistic regression was used to examine the prevalence of cartilage defects after adjusting for bone size, age, gender, BMI and MRI scanner. Cartilage volume was assessed by analysis of covariance (ANCOVA) adjusting for the same confounders as cartilage defects. All statistical analyses were performed on SPSS package (version 22.0, SPSS, Chicago, IBM) with significance accepted at P < 0.05.

Results

A total number of 130 participants were enrolled in the study. Three independent groups were examined: (i) isolated ACLR group without meniscal pathology (n = 62), (ii) combined group—ACLR plus meniscal pathology
(n = 38), and (iii) healthy control group (n = 30). In the combined group, a meniscal tear was present in 10 knees, but no surgical treatment was undertaken, seven participants had meniscal repair (4 of the medial meniscus and 3 of the lateral meniscus), and 21 participants had partial meniscectomy (8 medial meniscectomy, 12 lateral meniscectomy and 1 both medial and lateral meniscectomy). Surgeon recorded arthroscopic grade 2 cartilage defects at the time of ACLR: eight in the isolated ACLR group (13 %, 2 in medial femoral condyle, 1 in lateral tibia and 5 in patella) and nine in the combined group (24 %, 3 in the medial femoral condyle, 1 in the lateral femoral condyle and 5 in the patella; P = 0.28 for the difference between two ACLR groups). Only cartilage defects with cartilage loss were recorded; hence, ICRS grade 1 cartilage soft was not included.

The three groups of participants had similar age and gender profiles, with the exception of BMI, where the combined group exhibited a significantly higher BMI than the isolated ACLR and control groups (Table 1). All KOOS subscale scores were significantly different across groups (P < 0.001). The two surgical subgroups showed no significant KOOS subscale differences. Side-to-side differences in anterior knee laxity were significantly different amongst the three groups (P = 0.002). BMLs on the tibia were more prevalent in the surgical groups than the control group (Table 2). Compared with controls, the isolated ACLR group showed high BML scores on the lateral tibia (P < 0.001), whilst the combined group showed higher scores on the medial tibia and lateral tibia (medial tibia: P = 0.017; lateral tibia: P < 0.001).

**Cartilage defects**

Cartilage defects were more prevalent in the isolated ACLR group and combined group compared with the controls across the knee (P < 0.001, Table 2). Compared with controls, the isolated ACLR group showed a higher prevalence of cartilage defects on the medial femoral condyle and lateral femoral condyle, whilst the combined group showed higher prevalence on the medial femoral condyle, lateral tibia, lateral femoral condyle and patella. The combined group exhibited higher prevalence of cartilage defects on the medial femoral condyle than the isolated ACLR group (all comparison P < 0.05). After adjustment for confounders, the prevalence was significantly higher on the lateral tibia and patella for the combined group (lateral tibia: P = 0.005; patella: P = 0.006) than for the control group (Table 3). The combined group showed significantly higher prevalence of cartilage defects than the isolated ACLR group on the medial femoral condyle (P = 0.02) and patella (P = 0.02), but not on other sites (Table 4).
Table 2  Crude MRI structural measurement in the three groups

<table>
<thead>
<tr>
<th>Cartilage defects: number (%)</th>
<th>ACLR (n = 62)</th>
<th>Combined (n = 38)</th>
<th>Controls (n = 30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial tibia</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 0</td>
<td>55 (89 %)</td>
<td>32 (84 %)</td>
<td>28 (93 %)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Grade 1</td>
<td>6 (10 %)</td>
<td>3 (8 %)</td>
<td>2 (7 %)</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>1 (2 %)</td>
<td>2 (5 %)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>–</td>
<td>1 (3 %)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Grade 4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Medial femoral condyle</td>
<td>a,c</td>
<td>b,c</td>
<td>a,b</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Grade 0</td>
<td>47 (76 %)</td>
<td>18 (47 %)</td>
<td>29 (97 %)</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>7 (11 %)</td>
<td>6 (16 %)</td>
<td>1 (3 %)</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>8 (13 %)</td>
<td>9 (24 %)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>–</td>
<td>5 (13 %)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Grade 4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Lateral tibia</td>
<td>b</td>
<td></td>
<td></td>
<td>0.04*</td>
</tr>
<tr>
<td>Grade 0</td>
<td>30 (48 %)</td>
<td>10 (26 %)</td>
<td>11 (37 %)</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>21 (34 %)</td>
<td>15 (39 %)</td>
<td>17 (57 %)</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>10 (16 %)</td>
<td>12 (32 %)</td>
<td>2 (7 %)</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>1 (2 %)</td>
<td>1 (3 %)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Grade 4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Lateral femoral condyle</td>
<td>a,b</td>
<td></td>
<td></td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Grade 0</td>
<td>25 (40 %)</td>
<td>10 (26 %)</td>
<td>30 (100 %)</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>–</td>
<td>2 (5 %)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>34 (55 %)</td>
<td>24 (63 %)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>2 (3 %)</td>
<td>2 (5 %)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Grade 4</td>
<td>1 (2 %)</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Patella</td>
<td>a,b</td>
<td></td>
<td></td>
<td>0.005*</td>
</tr>
<tr>
<td>Grade 0</td>
<td>37 (60 %)</td>
<td>24 (63 %)</td>
<td>26 (87 %)</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>21 (34 %)</td>
<td>6 (16 %)</td>
<td>3 (10 %)</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>3 (5 %)</td>
<td>7 (18 %)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>1 (2 %)</td>
<td>1 (3 %)</td>
<td>1 (3 %)</td>
<td></td>
</tr>
<tr>
<td>Grade 4</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Any defects in the knee</td>
<td>43 (69 %) a</td>
<td>32 (84 %) b</td>
<td>3 (10 %) a,b</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>BMLs: number (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial tibia</td>
<td>b</td>
<td></td>
<td></td>
<td>0.02*</td>
</tr>
<tr>
<td>Grade 0</td>
<td>28 (45 %)</td>
<td>11 (29 %)</td>
<td>19 (63 %)</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>9 (15 %)</td>
<td>10 (26 %)</td>
<td>7 (23 %)</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>13 (21 %)</td>
<td>13 (34 %)</td>
<td>3 (10 %)</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>12 (19 %)</td>
<td>4 (11 %)</td>
<td>1 (3 %)</td>
<td></td>
</tr>
<tr>
<td>Medial femoral condyle</td>
<td></td>
<td></td>
<td></td>
<td>n.s.</td>
</tr>
<tr>
<td>Grade 0</td>
<td>51 (82 %)</td>
<td>32 (84 %)</td>
<td>28 (93 %)</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>8 (13 %)</td>
<td>3 (8 %)</td>
<td>2 (7 %)</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>3 (5 %)</td>
<td>2 (5 %)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>–</td>
<td>1 (3 %)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Lateral tibia</td>
<td>a,b</td>
<td></td>
<td></td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Grade 0</td>
<td>26 (42 %)</td>
<td>20 (53 %)</td>
<td>29 (97 %)</td>
<td></td>
</tr>
<tr>
<td>Grade 1</td>
<td>12 (19 %)</td>
<td>6 (16 %)</td>
<td>1 (3 %)</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>13 (21 %)</td>
<td>6 (16 %)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>11 (18 %)</td>
<td>6 (16 %)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Lateral femoral condyle</td>
<td></td>
<td></td>
<td></td>
<td>n.s.</td>
</tr>
<tr>
<td>Grade 0</td>
<td>50 (81 %)</td>
<td>35 (92 %)</td>
<td>27 (90 %)</td>
<td></td>
</tr>
</tbody>
</table>
In crude analysis, compared with controls, the isolated ACLR group had significantly less cartilage volume on the medial tibia and patella ($P = 0.016$ and $P = 0.011$), whilst the combined group had less cartilage volume on the patella ($P = 0.012$, Table 2). After adjusting for confounders, cartilage volume was significantly lower for both the isolated ACLR group (medial tibia: $P < 0.001$; lateral tibia: $P = 0.001$; patella: $P = 0.002$) and the combined group (medial tibia: $P < 0.001$; lateral tibia: $P = 0.002$; patella: $P = 0.008$) compared with controls (Table 5; Fig. 1). Cartilage volume was not significantly different between the two surgical groups.

**Table 2** continued

<table>
<thead>
<tr>
<th></th>
<th>ACLR ($n = 62$)</th>
<th>Combined ($n = 38$)</th>
<th>Controls ($n = 30$)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1</td>
<td>7 (11 %)</td>
<td>3 (8 %)</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>Grade 2</td>
<td>4 (7 %)</td>
<td>–</td>
<td>2 (7 %)</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>1 (2 %)</td>
<td>–</td>
<td>1 (3 %)</td>
<td></td>
</tr>
<tr>
<td>Cartilage volume ($\text{mm}^3$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial tibia</td>
<td>2164.1 (±651.4)$^a$</td>
<td>2213.7 (±595.4)</td>
<td>2513.9 (±690.6)$^a$</td>
<td>0.048*</td>
</tr>
<tr>
<td>Lateral tibia</td>
<td>2920.8 (±845.7)</td>
<td>2879.6 (±796.9)</td>
<td>3145.4 (±879.5)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Patella</td>
<td>3560.6 (±1043.1)$^a$</td>
<td>3516.5 (±637.2)$^b$</td>
<td>4121.2 (±1149.5)$^{ab}$</td>
<td>0.02*</td>
</tr>
<tr>
<td>Measures of bone size</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial tibial bone area ($\text{mm}^2$)</td>
<td>2222.6 (±335.2)</td>
<td>2327.4 (±300.1)</td>
<td>2289.1 (±356.7)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Lateral tibial bone area ($\text{mm}^2$)</td>
<td>1313.0 (±209.1)</td>
<td>1289.2 (±186.4)</td>
<td>1296.5 (±221.8)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Patellar bone volume ($\text{mm}^3$)</td>
<td>20,106.4 (±4856.0)</td>
<td>19,930.5 (±3825.0)</td>
<td>19,756.6 (±4605.2)</td>
<td>n.s.</td>
</tr>
</tbody>
</table>

Data reported as mean (±standard deviation) or number (%). BMLs were not available in the patella because of lack of PD-weighted images in the sagittal plane.

**Table 3** Prevalence of cartilage defects in isolated ACLR and combined groups compared with controls

<table>
<thead>
<tr>
<th></th>
<th>Odds ratios (95 % CI)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lateral tibia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>1.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>ACLR</td>
<td>4.9 (0.9-25.5)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Combined</td>
<td>12.7 (2.1-76.4)</td>
<td>0.005*</td>
</tr>
<tr>
<td>Patella</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Controls</td>
<td>1.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>ACLR</td>
<td>4.2 (0.4-41.7)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Combined</td>
<td>31.6 (2.7-369.8)</td>
<td>0.006*</td>
</tr>
</tbody>
</table>

Adjusted for age, gender, BMI, MRI scanner and bone size. 95 % CI, 95 % confidence interval

* Significant difference ($P < 0.05$). The prevalence cartilage defect was defined as a score of ≥2 that was present in at least two consecutive MRI slices. $P$ values were only presented at lateral tibia and patella, because the control group showed no cartilage defect ≥2 at the medial tibia, medial femoral condyle and lateral femoral condyle.

**Table 4** Prevalence of cartilage defects in the combined group compared with the isolated ACLR group

<table>
<thead>
<tr>
<th></th>
<th>Odds ratios (95 % CI)</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial tibia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACLR</td>
<td>1.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Combined</td>
<td>8.5 (0.3-239.7)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Medial femoral condyle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACLR</td>
<td>1.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Combined</td>
<td>4.7 (1.3-16.6)</td>
<td>0.02*</td>
</tr>
<tr>
<td>Lateral tibia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACLR</td>
<td>1.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Combined</td>
<td>2.9 (0.9-8.7)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Lateral femoral condyle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACLR</td>
<td>1.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Combined</td>
<td>1.4 (0.5-3.8)</td>
<td>n.s.</td>
</tr>
<tr>
<td>Patella</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ACLR</td>
<td>1.0</td>
<td>n.s.</td>
</tr>
<tr>
<td>Combined</td>
<td>7.8 (1.5-40.7)</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

Adjusted for age, gender, BMI, MRI scanner and bone size. 95 % CI, 95 % confidence interval

* Significant difference ($P < 0.05$). The prevalence cartilage defect was defined as a score of ≥2 that was present in at least two consecutive MRI slices.
The most important finding of the present study was that both ACLR groups exhibited more TFJ and PFJ cartilage defects and less tibial and patellar cartilage volume at 2–3 years following surgery than controls. Furthermore, the combined group demonstrated a higher prevalence of cartilage defects on the medial femoral condyle and patella compared to their isolated ACLR counterparts. The ACLR cohorts exhibited greater anterior knee laxity than controls and were still experiencing some symptoms and activity-related limitations. However, they were representative of the wider ACLR population given that their KOOS scores were comparable to those of other studies that have tested ACLR patients with similar characteristics, such as time from ACL injury to surgery, time since surgery, ACL graft type, and age and gender distribution [7, 10].

Cartilage defects in the medial TFJ, lateral TFJ and PFJ were more prevalent in both ACLR groups compared with the control group. Consistent with the notion that defects are precursors of cartilage degeneration with more severe defects associated with faster cartilage loss [8], adjusted medial tibial, lateral tibial and patella cartilage volume of ACLR individuals were attenuated compared to their uninjured counterparts.

Both ACLR groups demonstrated comparatively more cartilage defects and smaller cartilage volume in the lateral TFJ than those of controls. This finding helps explain the disproportionately higher prevalence of degenerative changes identified in the lateral TFJ in the subsequent years following ACL injury compared with those at the medial TFJ [42]. The increased risk of cartilage defects in the lateral compartment is largely explained by the initial impact pattern of ACL injury. Noncontact ACL injury typically results from valgus and internal rotation and loading of the tibia [30]. Immediately following ACL rupture, the tibia subluxates anteriorly, a movement that causes the lateral femoral condyle to impact against the lateral tibial plateau [40]. As such, the lateral tibia is typically characterized by a higher number of acute BMLs in ACL-injured and ACLR populations [35, 40]. In support, we found a higher prevalence of BMLs on the lateral tibia in both ACLR groups compared with controls. Due to the damage to the cartilage and subchondral bone, post-traumatic OA is evenly distributed between the medial and the lateral compartments, which is a different pattern of disease development from nontraumatic OA that is primarily located in the medial compartment [42].

In the medial TFJ, both ACLR groups demonstrated more cartilage defects on the medial femoral condyle than those of controls. Quantitative measurements have detected cartilage thickening on the medial compartment [15, 16]. In the current study, we found smaller cartilage volume in both ACLR groups in the medial tibia compared with controls. Due to the damage to the cartilage and subchondral bone, post-traumatic OA is evenly distributed between the medial and the lateral compartments, which is a different pattern of disease development from nontraumatic OA that is primarily located in the medial compartment [42].

<table>
<thead>
<tr>
<th></th>
<th>ACLR (n = 62)</th>
<th>Combined (n = 38)</th>
<th>Controls (n = 30)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial tibia</td>
<td>2171.5 (±57.9)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2065.1 (±78.9)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2686.8 (±92.8)&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Lateral tibia</td>
<td>2860.8 (±71.6)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2825.1 (±99.1)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3338.3 (±114.7)&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>0.002*</td>
</tr>
<tr>
<td>Patella</td>
<td>3552.2 (±94.3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3536.9 (±129.9)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>4112.5 (±150.9)&lt;sup&gt;b,c&lt;/sup&gt;</td>
<td>0.007*</td>
</tr>
</tbody>
</table>

Data are presented as mean (±standard error)

<sup>*</sup>Significant difference (P < 0.05)
<sup>a</sup>Cartilage volume is adjusted for age, gender, BMI, MRI scanner and bone size
<sup>b</sup>Post hoc is significantly different for isolated ACLR versus controls
<sup>c</sup>Post hoc is significantly different for combined group versus controls
<sup>d</sup>Post hoc is significantly different for isolated ACLR versus combined group (P < 0.05)
alter medial compartment loading following ACLR. Specifically, early stance medial compartment loads during walking are generated predominantly by the hamstrings [50]. Hence, impaired medial hamstring force production may contribute to shifts in the location and size of contact stresses in the medial compartment.

In addition to the TF compartments, there is increasing recognition that the PFJ is also susceptible to the development of knee OA [6]. Relative to controls, both ACLR groups in our study had smaller patellar cartilage volume. Cartilage defects were more prevalent in the combined group than in controls, whilst no difference was found between the isolated ACLR group and controls. Our results suggest that PFJ cartilage, like that in the TFJ, is also susceptible to degenerative change following ACLR.

Meniscal injury and meniscectomy are well-established risk factors for the development of knee OA. Therefore, it is not surprising that the additional meniscal pathology, which was either surgically or nonsurgically treated, in ACLR patients accelerates the onset of knee OA in the years following ACLR [1, 23, 29, 32, 33]. Compared with an isolated ACL injury, the presence of the meniscal pathology likely augmented the risk of cartilage injury at the medial femoral condyle and patella, leading to a higher incidence of OA. A previous review concluded that the prevalence of OA in knees after an isolated ACL injury was 0–13 %, whilst this figure increased to 21–48 % with combined meniscal injuries [33]. The combined group exhibited a large confidence interval for the prevalence of cartilage defects, possibly reflecting greater heterogeneity of the participants and the fact that the management of meniscal tears depends on many factors including the age of the patient, location and type of the tear and symptoms [14].

Current results may have been compounded by the fact that our combined group included those with meniscal injury, meniscal repair and partial meniscectomy. In support of our grouping strategy, a recent study found no difference in cartilage T2 values between patients with ACLR plus meniscal repair and patients with ACLR plus partial meniscectomy [26]. This suggests that cartilage status may not be greatly different after meniscal repair or partial meniscectomy at midterm (i.e. 2–4 years) post-surgery. Our combined group exhibited more prevalent cartilage defects compared with isolated ACLR group, which may lead to augmented degenerative changes in the future.

To our knowledge, this is the first study to explore quantitative cartilage volume in ACLR patients with and without concomitant meniscal pathology. Our study has limitations. First, we used a cross-sectional study design so cause and effect cannot be inferred. The lack of MRI data on pre-surgery knee structure means that we cannot exclude the possibility that between-group differences were pre-existing. Comparisons between arthroscopic and post-surgical MRI grades of cartilage defects were not performed, given that use of different diagnostic methods may impact sensitivity, specificity and accuracy [37]. Second, we were only able to recruit ACL-injured participants following ACLR; hence, it is not possible to elucidate whether poorer cartilage status was attributed to the ACL injury or altered knee biomechanics after surgery. Therefore, it would be worthwhile comparing our results with those of ACL-injured patients who have not undergone surgical management. Whilst our study focused on cartilage morphology, biomechanical and biochemical factors contributing to these cartilage results need to be explored.

**Conclusion**

At 2–3 years following ACLR, cartilage at the TFJ and PFJ exhibited more defects and smaller volume compared with that of the uninjured healthy individuals. Moreover, ACLR with concomitant meniscal injury had a more pronounced detrimental effect on cartilage morphology than isolated ACLR. Current findings suggest that ACLR patients with associated meniscal pathology are subject to worse cartilage features than isolated ACLR patients, leading to a greater risk of OA in the long term. This observational study provided valuable information about joint health following the ACLR that increases understanding of joint degeneration in this cohort. The findings suggest that clinicians should counsel patients about the long-term joint sequelae of their ACL injury so that they can make informed decisions about potential OA preventive strategies.

**Acknowledgments** This study was supported by the National Health and Medical Research Council (NHMRC, project Grant 628850).

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of interest.

**References**


Chapter 5: Knee structural change 2 to 4 years following ACLR with and without combined meniscal injury

Abstract:

Purpose: The primary aim was to investigate the 2-year change in knee joint morphology (cartilage volume, cartilage defects and bone marrow lesions, BMLs) in individuals who have undergone anterior cruciate ligament reconstruction (ACLR) with or without meniscal pathology and in healthy controls. The secondary aim was to examine whether baseline cartilage defect and BML scores were associated with cartilage volume change in the ACLR individuals.

Method: 66 participants aged 18 - 40 years (32 with isolated ACLR, 25 with combined ACLR and meniscal pathology, and 9 healthy controls) underwent knee MRI at baseline (approximately 2.5 years post-surgery) and 2 years later. Cartilage volume (on the medial tibia, lateral tibia and patella), cartilage defects (on the medial tibia, medial femoral condyle, lateral tibia, lateral femoral condyle and patella) and BMLs (on the medial tibia, medial femoral condyle, lateral tibia and lateral femoral condyle) were assessed from MRI using validated methods.

Results: Cartilage volume increased significantly over 2 years for both ACLR groups in all cartilage sites (medial tibia, lateral tibia and patella, all $P < 0.05$), while the control group showed an increase in cartilage volume only in the medial tibia ($P = 0.003$). The isolated ACLR group showed significantly greater annual increase in lateral tibia and patella cartilage volume compared with the controls (lateral tibia $P = 0.03$ and patella $P = 0.01$) and compared with the combined ACLR group (lateral tibia $P = 0.05$ and patella $P = 0.04$). Patellar cartilage defects of the isolated ACLR group and medial tibial BMLs of the combined group regressed (improved) over the 2 years (cartilage defects $P = 0.02$; BMLs $P = 0.03$). The number of participants showing
progression (worsening) of cartilage defects and BMLs did not differ between the three groups, while a greater number of participants in the ACLR groups showed regression of lateral tibial BMLs compared with controls ($P = 0.04$). In ACLR individuals, baseline cartilage defects score was positively associated with the increase in cartilage volume in the lateral tibia ($B = 55.2; 95\% \text{ CI} 23.9, 86.4; P = 0.001$) and patella ($B = 50.1; 95\% \text{ CI} 10.2, 90.0; P = 0.02$), while baseline BMLs score was inversely related to the cartilage volume increase in the medial tibia ($B = -31.4; 95\% \text{ CI} -50.5, -12.3; P = 0.002$)

Conclusion: The results demonstrated differences in joint morphology changes over two years depending on the morphological feature, the group and the region examined. Baseline cartilage defects scores and BMLs scores are associated with cartilage volume change in this post-traumatic cohort.
5.1 Introduction

Osteoarthritis (OA) is a leading cause of pain and disability, and joint injuries are associated with early onset of the disease (Lohmander et al., 2007). Anterior cruciate ligament (ACL) injury is one of the most common knee injuries primarily affecting young active individuals, and ACL reconstruction (ACLR) is frequently performed to restore knee function (Spindler et al., 2008). Although ACLR improves knee stability, it does not appear to reduce the likelihood of OA development such that the prevalence of OA is around 30% in individuals who have undergone this procedure 10 years previously (Ajuied et al., 2014; Chalmers et al., 2014; Claes, 2012; Lohmander et al., 2007). Moreover, concomitant meniscal injury frequently occurs at the time of the ACL injury, and further elevates the risk of OA (Keays et al., 2010; Lohmander et al., 2007). It has been reported that people with ACLR and associated meniscal injuries have 2-4 times higher prevalence of OA compared with those with isolated ACL injury (Oiestad et al., 2009). While the mechanisms underpinning the development of subsequent OA are not fully elucidated, residual instability, altered joint-loading patterns and inflammatory processes are likely contributors (Dahlberg et al., 1994; Gokeler et al., 2013; Ristanis et al., 2005). The assessment of early joint changes may help further elucidate the aetiology/pathophysiology of post-traumatic OA and identify risk factors for disease onset and progression. Given the differences in OA risk following ACLR with and without combined meniscal pathology, a comparison of structural changes in these groups over time is warranted.

As discussed in Chapter two, MRI provides a sensitive and noninvasive method to obtain accurate and detailed information about joint morphology including cartilage volume/thickness, cartilage defects and BMLs (Eckstein et al., 2006). Recent MRI studies have revealed alterations in joint morphology in the early years following ACL injury irrespective of whether the injury is managed conservatively or with ACLR (Potter et al., 2012; Van Ginckel et al., 2013). With respect to cartilage morphology,
longitudinal studies using quantitative MRI measurements have revealed increases in
cartilage volume or thickness from 1 to 5 years after the initial ACL injury, that are more
pronounced in the medial compartment (Eckstein et al., 2015; Frobell, 2011; Frobell et
al., 2009; Su et al., 2013). This increase may reflect cartilage swelling or cartilage
hypertrophy resulting from increased water content or augmented cartilage matrix
synthesis (Eckstein et al., 2015). Cartilage defects represent early pathology following
joint injury that can be assessed on MRI generally using semi-quantitative rating scales
(Bekkers et al., 2009; Ding, Garnero, et al., 2005). A study with an 11-year follow-up
showed progressive worsening of cartilage defects in all knee compartments following
ACLR (Potter et al., 2012). Although cartilage degeneration is the central pathology in
OA, BMLs located in the subchondral bone are also believed to play an important role in
the pathogenesis of OA (Bennell et al., 2010; Felson et al., 2003; Hunter et al., 2006).
BMLs are frequently found immediately after the ACL injury as a ‘footprint’ of the
initial trauma (Patel et al., 2014) but exhibit a changeable natural history thereafter
(Hanypsiak et al., 2008; Papalia et al., 2015). It appears that while most post-traumatic
BMLs resolve over the first 1-2 years after the injury, new BMLs also develop during the
same period (Faber et al., 1999; Frobell, 2011; Frobell et al., 2008). It is therefore
apparent that substantial changes in joint morphology occur in the early years following
ACL injury. While not directly studied, given the increased risk of OA following ACL
and combined meniscal injury, it is likely that changes will be greater in this group
compared with those with isolated ACL injury.

There is also interest in understanding the association between different joint
morphologic features on MRI given that they may reflect different mechanisms and
stages in OA pathogenesis. As cartilage defects and BMLs are thought to represent
early structural abnormalities, these may predict the subsequent changes in cartilage
volume/thickness.
In summary, current understanding of early joint change and OA-related progression after ACLR is still relatively limited. Thus, comparisons of changes in MRI features between ACLR and control individuals and between those with isolated ACLR and those with ACLR combined with meniscal pathology will help elucidate the natural history of post-traumatic OA. In addition, given the potential detrimental effect of cartilage defects and BMLs in OA, it is important to explore whether these are similarly associated with cartilage volume change in the ACLR population.

5.1.1 Study aims and hypotheses

The primary aim of the research was to investigate the 2-year change in knee cartilage volume in: (i) participants with isolated ACLR, (ii) participants with ACLR combined with meniscal pathology, and (iii) healthy control participants.

Related to this primary aim, the following hypotheses were examined:

H1: Both ACLR groups will show increases in cartilage volume whilst the control group will show no change;

H2: Both ACLR groups will exhibit greater increase in cartilage volume compared with control participants; and

H3: The combined ACLR group will show greater increase in cartilage volume compared with the isolated ACLR group;

There were two secondary aims of the research. First, to investigate the 2-year change in other knee joint morphologic features, namely knee cartilage defects and BMLs in the three groups. Second, to examine whether baseline BMLs and cartilage defects scores are associated with the magnitude of cartilage volume change over 2 years in the ACLR individuals.

Related to the secondary aims, the following hypotheses were examined:
H₄): Both ACLR groups will exhibit progression (worsening) of cartilage defects and changes in BMLs whilst the control group will show no change;

H₅): Both ACLR groups will exhibit greater progression of cartilage defects and greater changes in BMLs compared with control participants;

H₆): The combined ACLR group will show greater progression of cartilage defects and greater changes in BMLs compared with the isolated ACLR group; and

H₇): Higher cartilage defects and BMLs score at baseline will be correlated with greater increase in cartilage volume over the 2 years.
5.2 Methods

5.2.1 Participants

Participants were recruited from October 2011 to November 2012 in Melbourne and the Gold Coast, Australia. The detailed recruitment process has been described in Chapter 3 (section 3.2.1). Inclusion criteria for ACLR participants were: (i) aged 18-40 years (with the upper age limit to reduce the likelihood of pre-existing OA); (ii) ACLR performed for an acute ACL tear within 6 months of injury; (iii) ACLR performed using semitendinosus and gracilis (hamstring) autograft; (iv) ACLR performed 2-3 years previously. Participants with ACLR were excluded if they had: (i) International Cartilage Repair Society (ICRS) cartilage defects grade > 2 noted at time of ACLR or osteophytes on arthroscopy or X-ray at baseline; (ii) other musculoskeletal, cardiovascular or neurological conditions; (iii) previous ACL surgery or subsequent knee surgery on the involved leg; (iv) body mass index (BMI) > 34 kg/m² because of unreliability of gait assessment collected for a separate study; (v) contraindications to MRI. Eligible participants who had concomitant meniscal pathology (i.e. meniscal injury, meniscal repair and partial meniscectomy) at the time of ACLR were assigned to the combined ACLR and meniscal pathology group. Healthy participants were recruited from associated universities using the following inclusion criteria: i) aged 18 - 40 years and ii) BMI <34 kg/m2. The exclusion criteria for healthy controls were: i) prior knee surgery, ii) known lower limb injury or abnormality, and iii) contraindications to MRI. The University of Melbourne and Griffith University Human Research Ethics Committees approved this study and all participants provided written informed consent.

5.2.2 Surgery and rehabilitation procedure

All ACLR were performed arthroscopically using the hamstring tendon autografts. The indications for the meniscal repair or partial meniscectomy procedure were based on the appearances of the tear at the time of surgery as determined by the surgeon. Partial meniscectomy was performed in participants with non-repairable meniscal injuries that
were deemed to be potentially symptomatic. No chondral surgery was undertaken as all lesions were less than International Cartilage Repair Society (ICRS) score grade 3.

The rehabilitation protocol emphasized rapid restoration of knee range of motion and quadriceps function. Most patients had resumed full weight bearing by 2 to 3 weeks and were riding a stationary bicycle by week 4 in order to strengthen their quadriceps and hamstring muscles. At 3 to 4 months, the majority of patients were running, followed by the commencement of sports-specific drills from 4 months.

5.2.3 Anthropometric and Clinical Assessments

Height, weight and BMI

Weight was measured to the nearest 0.1 kg using electronic scales. Height was measured to the nearest 0.1 cm using a stadiometer. Weight and height were used to calculate body mass index (BMI; kg/m²).

Sport activity level

The sports activity rating scale from the Cincinnati knee rating system was used to assess the activity level of the participants by considering both the frequency of play and the type of sports (Noyes et al., 1989). Higher scores indicate higher level of sports participation (0-100).

MRI assessment

MRI scan of the study knee was performed at baseline (approximately 2.5 years post-ACLR) and at follow-up 2 years later using whole body MRI units in Melbourne (3.0T, Siemens Magenetom Verio, Erlangen, Germany) and the Gold Coast (1.5T, GE Healthcare Signa, Wisconsin, USA). Knees were imaged using T1-weighted 3D gradient recall (Cicuttini et al., 1999) in the sagittal plane and proton density (PD)-weighted fat-saturated spin echo acquisition in the coronal plane. The MRI technical parameters used in Melbourne included: T1-weighted, flip angle 10 degrees; repetition time 12.5 ms;
echo time 4.9 ms; field of view 16 cm; slice thickness 1.5 mm; 60 partitions; 512 × 512 matrix; acquisition time 6 min 58 sec; PD-weighted, flip angle 155°, repetition time 2640 msec, echo time 37 msec, slice thickness 3 mm, field of view 16 cm, pixel matrix 256 × 256, acquisition time 1 min 55 sec. At the Gold Coast, these included: flip angle 55 degrees; repetition time 44 ms; echo time 12 ms; field of view 16 cm; slice thickness 1.5 mm; 256 × 256 matrix; acquisition time 11 min 56 sec; PD-weighted, flip angle 155°, repetition time 4000 msec, echo time 50 msec, slice thickness 3 mm, field of view 16 cm, pixel matrix 256 × 256, acquisition time 5 min 26 sec.

Cartilage volume was measured in the medial tibia, lateral tibia and patella using a method described in previous studies (Cicuttini et al., 1999; Wang et al., 2015). T1-weighted images were transferred to the software Osiris (University of Geneva, Switzerland). Cartilage segmentation involved tracing the bone interface and the cartilaginous joint surface slice-by-slice. A trained observer (XW) who was blinded to the participants’ characteristics performed all MRI measures. The intra-rater reliability was assessed on two occasions with a time interval of 7 days, and the ICCs were 0.995, 0.996 and 0.997 for the cartilage volume on the medial tibia, lateral tibia and patella, respectively. The inter-rater reliability was determined prior to the assessment between the observer and an experienced radiologist, and the ICCs were 0.985, 0.987 and 0.993 for medial tibia, lateral tibia and patella, respectively. Annual change was determined by: (cartilage volume at follow-up – cartilage volume at baseline)/ time between MRI scans. Thus positive values represent an increase in cartilage volume.

Cartilage defects were graded in the medial tibia, medial femoral condyle, lateral tibia, lateral femoral condyle and patella using the T1-weighted image. One examiner with over 8 years experience assessed cartilage defects using the ICRS score as follows: grade 0, normal cartilage; grade 1, focal blistering and intra-cartilaginous low-signal intensity area with an intact surface and base; grade 2, irregularities on the surface or base thickness < 50%; grade 3, deep ulceration with loss of thickness > 50%; grade 4,
full-thickness cartilage wear with exposure of subchondral bone (Ding et al., 2005). A cartilage defect had to be present in ≥ 2 consecutive slices. Intra-observer and inter-observer agreement (expressed as Intra-class correlation coefficients, ICCs) values were 0.89-0.94 and 0.85-0.93, respectively. Cartilage defects was defined as ‘progression’ if the cartilage defect score increased (worsened), ‘regression’ if the cartilage defect score decreased (improved) or ‘stable’ if the cartilage defect score did not change (this included those who had no defect at baseline and did not develop a defect) over 2 years.

BMLs were examined on the PD-weighted fat-saturated images in the medial tibia, medial femoral condyle, lateral tibia and lateral femoral condyle. BMLs at the patella were not graded because MRI images were only available in the coronal plane. The size of BMLs was graded from 0 to 3 based on the extent of regional involvement in 10 subregions (the anterior, central, and posterior regions of medial and lateral tibia; and central and posterior regions of medial and lateral femur) as following: 0, none; 1, < 1/3 of the subregional volume; 2, 1/3 – 2/3 of the subregional volume; 3, > 2/3 of the subregional region) (Hunter et al., 2011). The intra- and inter-observer reliability (expressed as weighted kappa-values) in the reading of the BMLs ranged from 0.50-1.00 and 0.67-1.00. The score of BMLs was defined as the maximum score among the subregions in the subchondral bone. BMLs were defined as ‘progression’ if the BML score increased, ‘regression’ if the BML score decreased or ‘stable’ if the BML score did not change (this included those who had no BML at baseline and did not develop a BML) over 2 years.

5.2. Statistical analysis

Only participants with full datasets (i.e. baseline and follow-up measures) were included in the main analysis. Means and standard deviations are presented for continuous variables with normal distribution, while ordinal variables are shown by median and interquartile range. Paired T-test and Wilcoxon signed-rank test were used to examine the longitudinal change within each group. Annual change in cartilage volume was compared
between the three groups by analysis of covariance (ANCOVA) adjusting for age, gender, BMI, baseline cartilage defect and baseline bone size. Chi-squared test and Fisher exact test were used to explore group difference in change in cartilage defects and BMLs (progression and regression). In the event of a significant main effect, post hoc comparisons were conducted if necessary using the Fisher's least significant difference (LSD) test or Mann-Whitney U test. Logistic regression was used to examine the risk of progression in cartilage defects and BMLs between ACLR with or without meniscal injury after adjusting for age, gender and BMI. The association between predictors (cartilage defects and BML) and cartilage volume change were examined using univariate and multivariate linear regression with the ACLR groups amalgamated. All statistical analyses were performed using SPSS package (version 22.0, SPSS, Chicago, IBM) with significance accepted at P < 0.05.
5.3 Results

5.3.1 Participants

*Characteristics*

Three groups with baseline and follow-up measures were examined: i) isolated ACLR group (n = 32); ii) combined ACLR group (n = 25) and iii) control group (n = 9). The characteristics comparing the three groups are shown in Table 5.1. In the combined group, 9 participants had a meniscal tear without surgical treatment (4 in medial meniscus and 5 in the lateral meniscus), 4 participants had meniscal repair (3 of the medial meniscus and 1 of the lateral meniscus), and 12 participants had partial meniscectomy (5 medial meniscectomy and 7 lateral meniscectomy). Participants in the combined group exhibited a significantly higher BMI than those in the isolated ACLR group (P = 0.008). The control group had a significantly longer time interval between baseline and follow-up than both surgical groups (P < 0.001). No differences were found for other variables.
Table 5.1 Characteristics of participants in three groups

<table>
<thead>
<tr>
<th></th>
<th>ACLR isolated (n = 32)</th>
<th>ACLR combined (n = 25)</th>
<th>Controls (n = 9)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>30.7 (± 6.4)</td>
<td>30.6 (± 7.1)</td>
<td>28.3 (± 4.0)</td>
<td>0.58</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>19 (59%)</td>
<td>18 (72%)</td>
<td>8 (89%)</td>
<td>0.24</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.4 (± 3.2)</td>
<td>27.0 (± 3.6)</td>
<td>24.6 (± 3.8)</td>
<td>0.02</td>
</tr>
<tr>
<td>Sports activity level at baseline</td>
<td>85 (80 95)</td>
<td>80 (75 91)</td>
<td>88 (85 88)</td>
<td>0.57</td>
</tr>
<tr>
<td>Sports activity level at follow-up</td>
<td>80 (80 95)</td>
<td>80 (70 95)</td>
<td>90 (78 98)</td>
<td>0.58</td>
</tr>
<tr>
<td>Time between assessments (yr)</td>
<td>2.1 (± 0.2)</td>
<td>2.0 (± 0.2)</td>
<td>2.9 (± 0.4)</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

Parametric data presented as mean (± standard deviation), and sports activity levels presented as median (interquartile range). BMI, body mass index. * Significant difference (P < 0.05). Post hoc was significantly different for ¹ isolated ACLR versus controls; ² combined group versus controls; ³ isolated ACLR versus combined group (P < 0.05).

Comparison between participants who returned for follow-up and those who withdrew

Five-seven (57%) out of 100 participants with ACLR and 9 (30%) out of 30 healthy controls returned for follow-up testing. For the ACLR participants, 21 could not be contacted, 11 did not return due to excessive time commitment, 5 had additional ACL or meniscal injury, 1 relocated and 5 had MRI-related issues such as pregnancy (n=2), wearing intrauterine device (n=1) and MRI scanner problem (n=2). For the control group, all those who withdrew from the study did so due to relocation (n=21). The characteristics were compared between those who completed the follow-up assessment and those who did not, for the ACLR participants (Table 5.2) and control participants (Table 5.3), respectively. There were no significant differences in either ACLR group or control group.
Table 5.2 Characteristics of ACLR participants who completed the study and those who were lost to follow-up

<table>
<thead>
<tr>
<th>Completed study (n = 57)</th>
<th>Lost to follow-up (n = 43)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at baseline (yr)</td>
<td>30.7 (± 6.7)</td>
<td>29.4 (± 6.3)</td>
</tr>
<tr>
<td>Gender, male (%)</td>
<td>37 (65%)</td>
<td>29 (67%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.6 (± 3.6)</td>
<td>24.8 (± 3.6)</td>
</tr>
<tr>
<td>Meniscal pathology, n (%)</td>
<td>25 (44%)</td>
<td>13 (30%)</td>
</tr>
<tr>
<td>Sports activity level at baseline</td>
<td>85 (80, 95)</td>
<td>88 (79, 95)</td>
</tr>
</tbody>
</table>

BMI, body mass index. Parametric data presented as mean (± standard deviation), and sports activity levels presented as median (interquartile range).

Table 5.3 Characteristics of control participants who completed the study and those who were lost to follow-up

<table>
<thead>
<tr>
<th>Completed study (n = 9)</th>
<th>Lost to follow-up (n = 21)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at baseline (yr)</td>
<td>28.3 (± 4.0)</td>
<td>28.4 (± 5.7)</td>
</tr>
<tr>
<td>Gender, male (%)</td>
<td>8 (89%)</td>
<td>11 (52%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.6 (± 3.8)</td>
<td>22.9 (± 3.0)</td>
</tr>
<tr>
<td>Sports activity level at baseline</td>
<td>88 (85, 88)</td>
<td>90 (75, 100)</td>
</tr>
</tbody>
</table>

BMI, body mass index. Parametric data presented as mean (± standard deviation), and sports activity levels presented as median (interquartile range).

5.3.2 Longitudinal change in cartilage volume

Two-year change in cartilage volume within each group

The longitudinal change in cartilage volume is shown in Table 5.4. Both ACLR groups exhibited a significant increase in cartilage volume at all cartilage sites (medial tibia, lateral tibia and patella, all \( P < 0.05 \)), while for the control group, a significant increase was found in the medial tibia only (\( P = 0.003 \)).
<table>
<thead>
<tr>
<th>Site</th>
<th>ACLR isolated (n = 32)</th>
<th></th>
<th>ACLR combined (n = 25)</th>
<th></th>
<th>Controls (n = 9)</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
<td>Mean change</td>
<td>P value</td>
<td>Baseline</td>
<td>Follow-up</td>
<td>Mean change</td>
<td>P value</td>
</tr>
<tr>
<td>Medial</td>
<td>2043.3</td>
<td>2134.3</td>
<td>91.0</td>
<td><strong>0.02</strong>*</td>
<td>2245.1</td>
<td>2354.6</td>
<td>109.4</td>
<td><strong>0.01</strong>*</td>
</tr>
<tr>
<td>tibia</td>
<td>(553.0)</td>
<td>(594.4)</td>
<td>(18.2,163.7)</td>
<td></td>
<td>(468.9)</td>
<td>(506.2)</td>
<td>(27.2,191.7)</td>
<td></td>
</tr>
<tr>
<td>Lateral</td>
<td>2669.1</td>
<td>2788.3</td>
<td>119.2(40.6, 197.7)</td>
<td><strong>0.004</strong></td>
<td>2918.7</td>
<td>3007.5</td>
<td>88.8</td>
<td><strong>0.04</strong>*</td>
</tr>
<tr>
<td>tibia</td>
<td>(696.0)</td>
<td>(724.1)</td>
<td>197.7</td>
<td>*</td>
<td>(795.7)</td>
<td>(835.4)</td>
<td>(3.0,174.6)</td>
<td></td>
</tr>
<tr>
<td>Patella</td>
<td>3328.2</td>
<td>3548.3</td>
<td>220.1(138.8, 301.3)</td>
<td><strong>&lt;0.00</strong></td>
<td>3470.0</td>
<td>3595.9</td>
<td>125.9(25.6, 226.2)</td>
<td><strong>0.02</strong>*</td>
</tr>
<tr>
<td></td>
<td>(822.0)</td>
<td>(854.2)</td>
<td>(555.4)</td>
<td></td>
<td>(634.3)</td>
<td>(634.3)</td>
<td>(835.6)</td>
<td></td>
</tr>
</tbody>
</table>

* Significant difference (P< 0.05). Cartilage volume change = follow-up - baseline, thus positive values represent an increase in cartilage volume.
Comparison of annual change in cartilage volume between groups

The adjusted annual change in cartilage volume in each group is shown in Table 5.5 and Figure 5.1. After adjustment for confounders, the isolated ACLR group showed significantly greater annual increase in cartilage volume than controls at the lateral tibia \((P = 0.03, \text{mean difference } 76.4 \text{ mm}^3, 95\% \text{ CI } 7.0, 145.8)\) and at the patella \((P = 0.01, \text{mean difference } 111.5 \text{ mm}^3, 95\% \text{ CI } 27.9, 195.1 \text{ mm}^3)\). There were no significant differences when comparing the combined ACLR group and controls at any site. Comparing the ACLR groups, the isolated ACLR group showed a significantly greater annual increase at the patella \((P = 0.04, \text{mean difference } 65.9 \text{ mm}^3, 95\% \text{ CI } 4.3, 127.5 \text{ mm}^3)\), while at the lateral tibia the increase just failed to reach statistical significance \((P = 0.052, \text{mean difference } 51.5 \text{ mm}^3, 95\% \text{ CI } -0.4, 103.3 \text{ mm}^3)\).

<table>
<thead>
<tr>
<th></th>
<th>ACLR isolated (n = 32)</th>
<th>ACLR combined (n = 25)</th>
<th>Controls (n = 9)</th>
<th>(P) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial tibia</td>
<td>53.4 (23.4, 83.4)</td>
<td>34.0 (-0.4, 68.4)</td>
<td>46.6 (-10.0,103.2)</td>
<td>0.71</td>
</tr>
<tr>
<td>Lateral tibia</td>
<td>70.6 (37.8,103.3)</td>
<td>19.4 (-18.3, 57.2)</td>
<td>-6.2 (-67.5,55.2)</td>
<td>0.04*</td>
</tr>
<tr>
<td>Patella</td>
<td>113.4 (74.3,152.4)</td>
<td>47.5 (2.3, 92.6)</td>
<td>1.9 (-72.2, 75.9)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Data presented as mean (95% confidence interval). * Adjusted for age, gender, BMI, baseline cartilage defect and baseline bone size. Post hoc testing was significantly different for \(^{1}\) isolated ACLR versus controls; \(^{2}\) combined group versus controls; \(^{3}\) isolated ACLR versus combined group \((P < 0.05)\).
5.3.3 Longitudinal change in cartilage defects

The median (interquartile range) of baseline and follow-up cartilage defect scores in each group are shown in Table 5.6. Cartilage defect scores in each group were unchanged over 2 years, with the exception of a significant decrease in score at the patella in the isolated ACLR group ($P = 0.02$).
The number (%) of participants who had progression, stable or regression of cartilage defects at each of the knee sites is shown in Table 5.7. The majority of participants in each group had stable cartilage defects although approximately 25% of both isolated and combined ACLR participants showed regression of defects in the patella.

There were no significant differences in the proportions of participants who had progression, stable or regression of cartilage defects when comparing the three groups at each site. After adjusting for confounders, the isolated ACLR group showed no difference in the risk of cartilage defect progression compared with the combined group (Table 5.8).

<table>
<thead>
<tr>
<th>Site</th>
<th>ACLR isolated (n = 32)</th>
<th>ACLR combined (n = 25)</th>
<th>Controls (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
<td>P value</td>
</tr>
<tr>
<td>Medial tibia</td>
<td>0 (0, 0)</td>
<td>0 (0, 0)</td>
<td>0.32</td>
</tr>
<tr>
<td>Medial femoral condyle</td>
<td>0 (0, 0)</td>
<td>0 (0, 0)</td>
<td>0.58</td>
</tr>
<tr>
<td>Lateral tibia</td>
<td>0 (0, 1)</td>
<td>0 (0, 1)</td>
<td>0.53</td>
</tr>
<tr>
<td>Lateral femoral condyle</td>
<td>0 (0, 2)</td>
<td>0 (0, 2)</td>
<td>0.32</td>
</tr>
<tr>
<td>Patella</td>
<td>0 (0, 1)</td>
<td>0 (0, 0)</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

* Significant difference (P < 0.05).
Table 5.7 Cartilage defects change at each site between the three groups given as n (%)

<table>
<thead>
<tr>
<th>Site</th>
<th>Cartilage defects changes</th>
<th>ACLR isolated (n = 32)</th>
<th>ACLR combined (n = 25)</th>
<th>Controls (n = 9)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Progression</td>
<td>1 (3%)</td>
<td>0 (0)</td>
<td>1.0</td>
</tr>
<tr>
<td>Medial tibia</td>
<td></td>
<td>Stable</td>
<td>31 (97%)</td>
<td>24 (96%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Regression</td>
<td>0 (0)</td>
<td>1 (4%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Progression</td>
<td>2 (6%)</td>
<td>4 (16%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stable</td>
<td>27 (84%)</td>
<td>20 (80%)</td>
<td>9 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Regression</td>
<td>3 (9%)</td>
<td>1 (4%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Progression</td>
<td>2 (6%)</td>
<td>0 (0)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Lateral tibia</td>
<td></td>
<td>Stable</td>
<td>25 (78%)</td>
<td>23 (92%)</td>
<td>9 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Regression</td>
<td>5 (16%)</td>
<td>2 (8%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Progression</td>
<td>1 (3%)</td>
<td>2 (8%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stable</td>
<td>31 (97%)</td>
<td>20 (80%)</td>
<td>9 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Regression</td>
<td>0 (0)</td>
<td>3 (12%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Patella</td>
<td></td>
<td>Progression</td>
<td>1 (3%)</td>
<td>2 (8%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stable</td>
<td>23 (72%)</td>
<td>17 (68%)</td>
<td>9 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Regression</td>
<td>8 (25%)</td>
<td>6 (24%)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
**Table 5.8 Risk of cartilage defect progression in the combined group compared with the isolated ACLR group**

<table>
<thead>
<tr>
<th>Compartment</th>
<th>ACLR isolated (n = 32)</th>
<th>ACLR combined (n = 25)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial tibiofemoral compartment</td>
<td>1</td>
<td>0.3 (0.02 – 3.1)</td>
<td>0.28</td>
</tr>
<tr>
<td>Lateral tibiofemoral compartment</td>
<td>1</td>
<td>0.6 (0.1 – 2.9)</td>
<td>0.51</td>
</tr>
<tr>
<td>Tibiofemoral joint combined</td>
<td>1</td>
<td>0.4 (0.1 – 1.6)</td>
<td>0.18</td>
</tr>
<tr>
<td>Patella</td>
<td>1</td>
<td>0.8 (0.2 – 3.0)</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Data presented as odds ratios (95% confidence interval). *Adjusted for age, gender and body mass index.

**5.3.4 Longitudinal change in bone marrow lesions**

The median (interquartile range) of baseline and follow-up BML scores in each group are show in Table 5.9. BML scores in each group remained unchanged over 2 years, with the exception of a significant score decrease in the combined ACLR group at the medial tibia ($P = 0.03$).
Table 5.9 Median (IQR) baseline and follow-up BML score with pre-post Wilcoxon test in each group

<table>
<thead>
<tr>
<th>Site</th>
<th>ACLR isolated (n = 32)</th>
<th>ACLR combined (n = 25)</th>
<th>Controls (n = 9)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Follow-up</td>
<td>P value</td>
</tr>
<tr>
<td>Medial tibia</td>
<td>1 (0, 2)</td>
<td>0 (0, 2)</td>
<td>1.0</td>
</tr>
<tr>
<td>Medial femoral condyle</td>
<td>0 (0, 0)</td>
<td>0 (0, 0)</td>
<td>0.93</td>
</tr>
<tr>
<td>Lateral tibia</td>
<td>1 (0, 2)</td>
<td>0 (0, 1.5)</td>
<td>0.06</td>
</tr>
<tr>
<td>Lateral femoral condyle</td>
<td>0 (0, 0)</td>
<td>0 (0, 0)</td>
<td>0.87</td>
</tr>
</tbody>
</table>

* Significant difference (P < 0.05).

The number (%) of participants who had progression, stable or regression of BMLs in each of the three groups is shown in Table 5.10. There were no significant differences in the proportions of participants between groups with progression, stable or regression of BMLs at any of the sites except at the lateral tibia (Table 5.10). At this site, a significantly greater number of participants in both ACLR groups showed regression of BMLs compared with the control group (isolated ACLR, $P = 0.01$; combined group, $P = 0.04$). However, there was no difference between the two ACLR groups at this site. After adjusting for confounders, the isolated ACLR group showed no difference in the risk of BMLs progression compared with the combined ACLR group in any compartment (Table 5.11).
Table 5.10 BMLs changes between the three groups given as n (%)

<table>
<thead>
<tr>
<th>Site</th>
<th>BML changes</th>
<th>ACLR isolated (n = 32)</th>
<th>ACLR combined (n = 25)</th>
<th>Controls (n = 9)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Progression</td>
<td>Stable</td>
<td>Regression</td>
<td></td>
</tr>
<tr>
<td>Medial tibia</td>
<td></td>
<td>9 (28%)</td>
<td>5 (20%)</td>
<td>0 (0)</td>
<td>0.25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stable</td>
<td>15 (47%)</td>
<td>9 (36%)</td>
<td>6 (67%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Regression</td>
<td>8 (25%)</td>
<td>11 (44%)</td>
<td>3 (33%)</td>
</tr>
<tr>
<td>Medial femoral condyle</td>
<td></td>
<td>Progression</td>
<td>3 (9%)</td>
<td>3 (12%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stable</td>
<td>25 (78%)</td>
<td>19 (76%)</td>
<td>9 (100%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Regression</td>
<td>4 (13%)</td>
<td>3 (12%)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Lateral tibia</td>
<td></td>
<td>Progression</td>
<td>7 (22%)</td>
<td>4 (16%)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>
|                       |             | Stable                 | 11 (34%)
1
|                       |             | Regression             | 14 (44%)
1
|                       |             | Progression            | 4 (13%)                | 5 (20%)         | 2 (22%) | 0.59    |
|                       |             | Stable                 | 23 (72%)               | 17 (68%)        | 6 (67%) | 0.94    |
|                       |             | Regression             | 5 (16%)                | 3 (12%)         | 1 (11%) | 1.0     |

Data presented as number (%). *Significant difference (*P* < 0.05). Post hoc was significantly different for 
1 isolated ACLR versus controls and 
2 combined group versus controls (*P* < 0.05). BMLs are not available in the patella because lack of PD-weighted images in the sagittal plane.

Table 5.11 Risk of BMLs progression in the combined ACLR group compared with the isolated ACLR group

<table>
<thead>
<tr>
<th>Compartment</th>
<th>ACLR isolated (n = 32)</th>
<th>ACLR combined (n = 25)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial tibiofemoral compartment</td>
<td>1</td>
<td>1.0 (0.3 – 3.4)</td>
<td>0.98</td>
</tr>
<tr>
<td>Lateral tibiofemoral compartment</td>
<td>1</td>
<td>0.9 (0.3 – 3.1)</td>
<td>0.91</td>
</tr>
<tr>
<td>Tibiofemoral joint combined</td>
<td>1</td>
<td>0.8 (0.3 – 2.3)</td>
<td>0.64</td>
</tr>
</tbody>
</table>

Data were presented as odds ratios (95% confidence interval). *Adjusted for age, gender and body mass index.
5.3.5 Relationship of baseline cartilage defect and BML scores with cartilage volume change in ACLR participants

Associations between baseline cartilage defects and BMLs scores with annual cartilage volume change were explored in ACLR participants pooled (n = 57). Before adjustment, baseline cartilage defects score was significantly and positively associated with the increase in cartilage volume in all cartilage sites (medial tibia $P = 0.005$, lateral tibia $P = 0.007$ and patella $P = 0.03$, Table 5.12). After adjustment for potential confounders, baseline cartilage defects scores in the lateral tibia and patella were positively associated with the increase in cartilage volume (lateral tibia: regression coefficient ($B$) = 55.2; 95% CI 23.9, 86.4; $R^2 = 0.3$; $P = 0.001$; patella: $B = 50.1$; 95% CI 10.2, 90.0; $R^2 = 0.2$; $P = 0.02$), while in the medial tibia the association just failed to reach statistical significance ($P = 0.053$; Table 5.12).

### Table 5.12 Association between baseline cartilage defects and cartilage volume change

<table>
<thead>
<tr>
<th></th>
<th>Univariate regression coefficient (95% CI)</th>
<th>$P$ Value</th>
<th>Multivariate regression coefficient (95% CI)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial tibia</td>
<td>91.9 (28.7, 155.2)</td>
<td>0.005*</td>
<td>62.7 (-0.8, 126.3)</td>
<td>0.053</td>
</tr>
<tr>
<td>Lateral tibia</td>
<td>42.4 (11.9, 73.0)</td>
<td>0.007*</td>
<td>55.2 (23.9, 86.4)</td>
<td>0.001*</td>
</tr>
<tr>
<td>Patella</td>
<td>43.7 (3.6, 83.9)</td>
<td>0.03*</td>
<td>50.1 (10.2, 90.0)</td>
<td>0.02*</td>
</tr>
</tbody>
</table>

Multivariate analysis was adjusted for age, BMI, baseline cartilage defects, baseline bone size and presence of meniscal pathology. *$P < 0.05$

In contrast, for BMLs, an unadjusted association was only found in the medial tibia where the baseline BML score was negatively associated with the annual cartilage
increase ($P = 0.02$, Table 5.13). After adjustment for potential confounders, baseline BMLs score was inversely related to the cartilage increase in the medial tibia ($B = -31.4$; 95% CI -50.5, -12.3; $R^2 = 0.4$; $P = 0.002$; Table 5.13).

Table 5.13 Association between baseline BMLs and cartilage volume change

<table>
<thead>
<tr>
<th></th>
<th>Univariate regression coefficient (95% CI)</th>
<th>$P$ Value</th>
<th>Multivariate regression coefficient (95% CI)</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medial tibia</td>
<td>-26.0 (-46.9, -5.0)</td>
<td>0.02*</td>
<td>-31.4 (-50.5, -12.3)</td>
<td>0.002*</td>
</tr>
<tr>
<td>Lateral tibia</td>
<td>-8.8 (-31.5, 13.7)</td>
<td>0.44</td>
<td>-16.3 (-38.5, 5.9)</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Multivariate analysis was adjusted for age, BMI, baseline BMLs, baseline bone size and presence of meniscal pathology. BMLs are not available in the patella because lack of PD-weighted images in the sagittal plane. *$P < 0.05$
5.4 Discussion

This study examined the two-year longitudinal change in knee joint morphologic features in ACLR individuals with or without combined meniscal pathology and the control group. Both ACLR groups showed cartilage volume increases at all measured sites while controls exhibited an increase only at the medial tibia. Cartilage defects remained stable for most participants while BMLs showed variable change. The results demonstrated differences in joint morphology changes between ACLR participants and controls but these changes depended on the morphological feature, the group and the joint region examined. Specifically, the isolated ACLR group exhibited greater annual increase in cartilage volume than controls in the lateral tibia and patella, whereas the combined group showed no difference at any site compared with controls. The isolated ACLR group also showed significantly greater increase in cartilage volume, but only at the patella, compared with the combined ACLR group. There was no difference in cartilage defect changes between the three groups. A greater number of participants in both ACLR groups showed regression (improvement) of BMLs at the lateral tibia when compared with controls. Baseline cartilage defects were positively associated with cartilage volume increase in the lateral tibia and patella, while baseline BMLs were negatively correlated with cartilage volume increase in the medial tibia in people with ACLR.

5.4.1 Longitudinal change in cartilage volume

In partial support of H1, both ACLR groups showed a significant increase in cartilage volume at all cartilage sites (medial tibia, lateral tibia and patella) from 2.5 to 4.5 years post-surgery. This concurs with several recent longitudinal studies that have also reported increased cartilage volume or thickness from 1 to 5 years following ACLR (Eckstein et al., 2015; Frobell, 2011; Frobell et al., 2009; Su et al., 2013). In contrast to established OA where reductions in cartilage volume/thickness is the characteristic of the disease progression (F. M. Cicuttini, Jones, et al., 2004; Wang et al., 2012), increases in cartilage volume or thickness have been reported in early OA populations (Buck et al., 2013; Buck
et al., 2010; Cotofana et al., 2012) as well as in animal models (Brandt et al., 1991; Calvo et al., 2001; Calvo et al., 2004) prior to cartilage loss. Thus, increased cartilage volume is suggestive of early cartilage degeneration, likely caused by cartilage hypertrophy or cartilage swelling (Dijkgraaf et al., 1995; Eckstein et al., 2015). The maintenance of cartilage morphology and function depends upon a delicate balance between swelling properties of the proteoglycans and counteracting collagen tension (Lehner et al., 1989; Maroudas, 1976). In the initial stage of cartilage degeneration, it has been suggested that the damaged collagen network does not adequately resist the swelling properties. As a result, the water content in cartilage increases and cartilage volume increases accordingly (Calvo et al., 2004; Martel-Pelletier et al., 2008). The increased cartilage volume could also reflect accelerated cartilage metabolism in an attempt to repair initial cartilage damage and withstand mechanical load (Eyre et al., 1980; Sandy et al., 1984).

Irrespective of the underlying mechanism, increased cartilage volume or thickness in this population appears to indicate impaired cartilage homeostasis and reduced mechanical ability (Eckstein et al., 2002; Maroudas, 1976) – factors thought to place the cartilage at a higher risk of more severe degenerative features including thinning and splitting (Dijkgraaf et al., 1995). Thus, the increased cartilage volume seen in the ACLR cohort is likely to be related to greater susceptibility to OA in the long-term.

Although it was hypothesised (H1) that there would be no change in cartilage volume in the control group, a significant increase was found in the medial tibia. This increase could represent adaptive change or the initial stage of degeneration due to mechanical loading. Biomechanical modeling data show that the medial compartment bears approximately 60% of the overall knee load during gait-related activities in healthy individuals (Winby et al., 2009) and it is well established that mechanical load influences articular cartilage structure (Wong et al., 2003). Indeed, in the same control cohort, our research group has found a moderate cross-sectional correlation ($R^2 = 0.3$) between medial tibiofemoral contact force during walking and medial tibial cartilage volume (Saxby et al., 2015). Alternatively, the increase in cartilage volume could be due to normal cartilage
development with age (Luria et al., 2014; Rieppo et al., 2009; Wei et al., 1998). However, this is unlikely given that the increase was confined to the medial tibia and was not seen across the other sites. Furthermore, whilst increased cartilage volume and thickening is observed amongst children and adolescents (Eckstein et al., 2014; Jones et al., 2003), our participants had a mean age of 28 years and had technically reached maturation.

5.4.2 Comparison of changes in cartilage volume between the three groups

The isolated ACLR group exhibited a greater annual increase in cartilage volume in the lateral tibia and patella compared with both the control group and with the combined ACLR group (H2). However, changes in cartilage volume in the combined ACLR group did not differ from controls. These results are somewhat counterintuitive on face value, as it was hypothesised that the combined group would demonstrate a more pronounced cartilage change (H2 and H3) given that concomitant meniscal pathology is the primary contributor to knee OA after ACLR (Oiestad et al., 2009). In support of this notion, recent compositional MRI studies using T2 relaxation time and T1 rho analyses reported greater cartilage degeneration amongst individuals with combined ACLR and meniscal pathology compared to those with isolated ACLR (Li et al., 2015; Li et al., 2011; Theologis et al., 2011). An explanation for the paradoxical findings might relate to the fact that cartilage volume change, as measured by the technique in this study, reflects change across the entire cartilage plate. Eckstein et al. (2014) identified simultaneous cartilage thickening and thinning in different sub-regions of the same cartilage plate after ACLR. Therefore, the final magnitude of cartilage volume change in one cartilage plate measured in the current study will depend upon the balance of cartilage thickening and thinning from all sub-regions (Eckstein et al., 2015). The isolated ACLR group experienced a greater cartilage volume increase than the other groups suggesting that on balance, increases were predominant across the cartilage plate subregions. In contrast, the combined group may have been undergoing a higher level of cartilage thinning in some subregions because of more advanced cartilage degeneration. This would partly negate
cartilage increases in other subregions resulting in less cartilage volume increase compared with the isolated group and similar increases compared to the control group. Further investigation using more specific assessment of cartilage subregions is warranted to confirm this postulation.

5.4.3 Longitudinal change in cartilage defects

Cartilage defect scores generally remained unchanged at most sites in the three groups over the 2-year timeframe. This was particularly evident in the controls where cartilage defects were stable at all sites. For the ACLR groups, the exception was the patellar site where the cartilage defect score showed a significant decrease in the isolated ACLR individuals with 25% participants showed regression (improvement) of defects. This suggests that the cartilage defects arise at the time of the initial injury and remain relatively stable from 2.5-4.5 years following ACLR at most sites except the patella. This is supported by an 11-year longitudinal study by Porter et al (Potter et al., 2012) that found cartilage defects in all 42 patients at the time of ACL injury, particularly in the lateral tibial plateau. Thereafter there was minimal change in cartilage defect size until approximately 7 years post injury when a marked increase was observed. It is likely that the increase at this latter time represents the acceleration of OA-degenerative processes and is consistent with the higher rates of OA observed around 10 years post ACL injury (Lohmander et al., 2007).

Interestingly, the current study found a regression of patellar cartilage defects in the isolated ACLR group. In a recent study with 111 participants one year following ACLR, Culvenor et al. (2015) reported that 17% participants had cartilage defects or osteophyte in the patellofemoral joint and they were categorised as MRI-defined patellofemoral OA (Culvenor et al., 2015). However, Zeng et al. (2015) suggested that the prevalence of OA was overestimated in this ACLR population as early as one year after surgery, and the prevalence of OA was reported as 28% and 20.3% over 10 years (Zeng et al., 2015; Claes et al., 2012; Ajuied et al., 2013). The current results suggest that the definition of OA
should be carefully selected, and the presence of cartilage defects seems to be inappropriate considering the regression of cartilage defects in some patients. There are conflicting data about the natural history of cartilage defects in population-based samples. In a younger cohort of 325 participants, largely without radiographic OA and with a mean age of 45 years, approximately 20% of participants showed an increase in cartilage defect grade over two years with similar numbers decreasing (Ding et al., 2006). In older age groups, regression of cartilage defects appears to be less common. For example, in a study of 395 participants with a mean age of 62.7 years, the average defect score significantly increased in all compartments over 2.9 years; however, the majority of defects remained stable and regression of defects was rare occurring in only 3.8% of participants (Carnes et al., 2012). In another study of 84 healthy participants with a mean age of 57 years, approximately two-thirds had an increase in knee cartilage defect grade while approximately 5% decreased in grade over 2 years (Wang et al., 2006). Less regression of cartilage defects in older compared with younger individuals could reflect declining mitotic and synthetic activity in chondrocytes that occurs with age in cartilage giving rise to less self-repair (Bhosale et al., 2008). Although articular cartilage is thought to have limited regeneration potential (Huey et al., 2012), the hypertrophic repair has been observed in the early stage of cartilage degeneration (Adams et al., 1991; Dijkgraaf et al., 1995). Furthermore, cartilage has been suggested to repair once the injury extends to subchondral bone, however the regenerated fibrocartilage exhibited inferior mechanical property than the normal hyaline cartilage (Buckwalter et al., 2004).

**5.4.4 Comparison of changes in cartilage defects between the three groups**

In contrast to H4 and H5, there was no difference in cartilage defects change between the three groups. This lack of difference between groups may be attributable to the: i) little change over the 2-years in cartilage defects (Section 5.4.3); ii) relatively small sample size with a lack of power to detect statistical differences. However, in the baseline study (Section 4.3.2), ACLR participants with combined meniscal pathology showed greater prevalence than those with isolated ACLR on lateral tibia and patella. The difference
between groups found at baseline is probably caused by a more severe impact at the time of initial ACL injury, as indicated by the meniscal injury.

5.4.3 Longitudinal change in BMLs

In general, BML scores remained unchanged over the 2 years in all participant groups at all sites, except for the combined ACLR group where a significant improvement was found in the medial tibia. Specifically, 44% of participants in the combined ACLR group exhibited BML regression over the 2-year period at this site. Similarly, BML regression has been reported in the tibiofemoral joint of 20% of knee OA patients over a two-year period (Kornaat et al., 2007). In addition, Roemer et al. (2009) reported that 50% of knee OA patients exhibited a decrease in the size of their BMLs over 30 months (Roemer et al., 2009). Unfortunately, neither Kornaat et al. (2007) nor Roemer et al. (2009) specified the location (i.e., medial or lateral compartment) of the BMLs.

BMLs are typically classified as traumatic or non-traumatic (Roemer et al., 2009). Trauma-induced BMLs are evident post-injury and are a direct result of high-force impact between articulating surfaces. By contrast, the pathogenesis of non-traumatic BMLs is less clear and interestingly, present in a variety of conditions including OA, vascular necrosis and inflammatory diseases including polyarthritis and osteomyelitis (Deangelis et al., 2010; Roemer et al., 2009).

Regression (improvement) of BMLs in the medial compartment of ACLR participants is likely influenced by joint loading (Beckwee et al., 2015). Indeed, a recent study by Wellsandt et al. (2016) demonstrated that during gait and sports-related activities, ACLR patients exhibit lower medial compartment contact forces in their involved limb (Wellsandt et al., 2016). Whilst the mechanistic pathways by which unloading alters the metabolism of the cartilage-subchondral bone unit leading to a resolution of BMLs is currently unclear, it may be related to changes in intra-osseous hypertension (Welch et al., 1993). BMLs could result in vascular penetration and an accumulation of inflammatory infiltrates (Binks et al., 2015). Given that intra-osseous pressure increases
in proportion to joint compressive load (Downey et al., 1988), underloading of the tibiofemoral joint – possibly driven by BML-related pain - may decrease pressure within the trabecular of the subchondral bone to a sufficient level to facilitate a gradual removal of inflammatory infiltrates. Importantly, increased intra-osseous pressure and inflammatory infiltrates could stimulate subchondral bone remodelling (Guilak et al., 2004; Welch et al., 1993). However, increased subchondral bone remodelling is a necessary condition for OA initiation, thus this may increase the risk of OA development in the ACL-injured patients (Burr et al., 2012; Li et al., 2013). Clearly, this theory requires substantiation via future research studies. Whilst the medial compartment is less prone to traumatic injury at the time of ACL rupture compared to the lateral compartment, many of them develop medial knee OA in the 10 years post-injury (Barenius et al., 2014; Li et al., 2011). Therefore, the presence of BMLs in the medial compartment is indicative of early joint abnormality, possibly attributed to the combined effect of altered loading and subsequent local inflammation.

With respect to the lateral compartment, the current study identified a resolution of BMLs in the lateral tibia of 44% and 36% of participants in the isolated and combined ACLR groups, respectively. That said, these results did not reach statistical significance. Previous studies have reported a resolution of 75%-80% of post-traumatic BMLs located in the lateral tibia and lateral femoral condyle, with the resolution process commencing in the first 3-6 weeks following ACL injury (Faber et al., 1999; Frobell et al., 2009; Szkopek et al., 2012). Interestingly, new BMLs have been found in the lateral compartment within the first 2 years post ACL injury (Frobell, 2011). Given that current study commenced at approximately 3 years after ACL rupture and MRI scans were not performed pre-surgery, it is somewhat difficult to differentiate whether BMLs in the lateral compartment are of a traumatic or non-traumatic nature.
5.4.4 Comparison of changes in BMLs between the three groups

In support of H₅, a greater number of participants in both ACLR groups showed regression of lateral tibial BMLs compared with the control group. The proportion of participants demonstrating progression, stable or regression in BMLs was similar between groups at other joint sites. This affirms the findings of other studies that post-traumatic BMLs resolve in the lateral knee compartment (Costa-Paz et al., 2001; Faber et al., 1999; Frobell, 2011; Frobell et al., 2008; Hanypsiak et al., 2008; Stein et al., 1995; Theologis et al., 2011). Meniscal pathology was found to increase the prevalence of BMLs in the OA population (Wang et al., 2010); however, the current study showed no difference in BML change between the two ACLR groups (in contrast to H₆). Non-significant differences between ACLR groups may be attributable to: i) the lack of statistical power given the relatively small sample size; ii) heterogeneity of meniscal pathology in the combined group whereby mild meniscal tears may not have the same impact on joint health as more severe meniscal tears, iii) measurement time frame, where differences may be more apparent with a longer time since surgery and/or, iv) measurement of BMLs using a semi-quantitative analyses which may lack sensitivity to change compared with a quantitative method (Frobell et al., 2009). Furthermore, the complexity of mechanisms contributing to BMLs should be considered since trauma, joint loading, physical activity are all associated with BMLs change (Beckwee et al., 2015; Lim et al., 2013).

5.4.5 Associations between baseline cartilage defects and cartilage volume change

Higher baseline cartilage defect scores in the lateral tibia and patella of ACLR participants were associated with greater cartilage volume increases over the subsequent two years. At baseline, cartilage defects were prevalent in the lateral tibia (60%) and patella (39%) amongst ACLR participants. The lack of association at the medial tibia is not surprising given the low prevalence of baseline cartilage defects (13%) in this region (see results reported in Section 4.3.2). It is noteworthy that the cartilage defects in the
current study were mild (ICRS grade 1-2), because ACLR patients with cartilage defects over grade 2 were excluded before the study.

The positive relationship between cartilage defects at baseline and cartilage volume increases over the subsequent two years indicates that more severe cartilage defects were associated with greater annual cartilage increase. As mentioned above (section 5.4.1), an increase in cartilage volume is probably suggestive of cartilage hypotrophy or swelling, indicative of early cartilage degeneration (Adams et al., 1991). Osteoarthritis studies in human and animal cartilage have reported an increase in the synthesis of proteoglycan and collagen as a result of ACL rupture, particularly in the early stages of OA development (Eyre et al., 1980; Floman et al., 1980; Sandy et al., 1984). A recent study also found an increase of aggrecan turnover in ACL injured patients, suggesting a localised cartilage repair response (Wasilko et al., 2015). From a mechanistic perspective, cartilage defects alter the normal distribution of loading in the knee joint and thereby, predispose the joint to degenerative changes (Strauss et al., 2011).

The current study demonstrated, for the first time, a quantitative relationship between cartilage defects and subsequent cartilage volume change in ACLR patients, however importantly, the results are limited to mild cartilage defects. Previous studies have been mainly focused on severe articular cartilage defects (grade 3-4), while the grade 1-2 cartilage defects are frequently referred to as ‘no chondral damage’ (Ichiba et al., 2009; Shelbourne et al., 2003). Severe cartilage damage was detected in 16-46% of patients at the time of ACLR and has been found to predict radiological OA development in ACLR cohorts (Brophy et al., 2010; Flanagan et al., 2010; Ichiba et al., 2009; Keays et al., 2010). Indeed, ACLR patients with severe cartilage defects reported worse knee function compared with those with mild or no cartilage defects at 2-5 years post-surgery (Rotterud et al., 2012; Rotterud et al., 2013; Shelbourne et al., 2003), and severe focal chondral lesions commonly require interventions such as microfracture, autologous chondrocyte implantation (ACI) and osteochondral autologous transplantation.
(OAT)(Bekkers et al., 2009). Although severe cartilage defects have been associated with OA-related outcomes, the current study exhibited that mild cartilage defects could also result in subsequent cartilage volume change. Clearly, there is a considerable need for additional research to develop more optimal treatment strategies for cartilage defects. At the very least, clinicians should take cartilage defects into consideration given the likelihood of long-term cartilage degenerative changes.

5.4.6 Associations between baseline BMLs and cartilage volume change

This is the first known study to examine the relationship between BMLs and change in medial tibia cartilage volume in an ACLR cohort. Higher scores for baseline BMLs in the medial tibia were associated with less cartilage volume increase over the two-year follow-up period. As mentioned above (see Section 5.4.3), the presence of BMLs in the medial compartment indicates early joint abnormality as a consequence of altered loading and/or local inflammation. From a mechanistic perspective, it may be that BMLs in the medial tibia impede the hypertrophic repair response of the overlying cartilage. In this respect, BMLs are suggested to reduce the stress-dissipating capacity of the cartilage-subchondral bone unit and, could also inhibit nutritional flow from the bone marrow to cartilage (Hunter et al., 2006).

By contrast, no significant associations were identified between BMLs and cartilage volume in the lateral tibia. These findings suggest that the medial and lateral cartilage-subchondral bone units respond quite differently when BMLs are present (see Section 5.4.3). With respect to the lateral compartment, several previous studies have reported no significant association between BMLs and cartilage morphology in the medium term (i.e., 2-3 years) following ACL injury (Frobell, 2011). By contrast, Potter et al. (2012) reported that greater traumatic BML size in the lateral tibiofemoral joint was associated with cartilage loss during the first 3 years following ACL injury, but not after that (Potter et al., 2012).
Whilst the results of studies examining the relationship between traumatic BMLs and subsequent cartilage volume change are not concordant, it appears that the association is clearer in radiographic knee OA patients with degenerative BMLs. Specifically, BMLs are associated with cartilage volume loss over two years in the OA patients, particularly in the medial compartment (Driban et al., 2011; Kothari et al., 2010; Raynauld, Martel-Pelletier et al., 2008; Roemer et al., 2009). In the current study, the negative association between baseline BMLs scores and cartilage volume change extends this idea to a younger ACLR cohort. Taken together, the presence of large size BMLs in the medial tibia could be a risk factor contributing to subsequent degenerative change in cartilage.

5.4.7 Strengths and Limitations

This study has several strengths. First, the study included a control group that was comparable in age and physical activity levels to the ACLR cohort. By contrast, other longitudinal studies of this nature have not included a control group to assess the natural history of cartilage morphological change. Second, ACLR participants with and without concomitant meniscal pathology were included. This is important given the large differences in the subsequent risk of early-onset knee OA in these groups. Third, this study incorporated a longitudinal design and included a number of measures of joint structure over a 2-year follow-up period.

The study also has several limitations. Only 51% of the participants returned for follow-up assessment despite considerable effort by the research team to minimise attrition. In this younger group with busy lives, high dropout was likely due to the time commitment required for biomechanical testing and MRI assessment (i.e., approximately 4 hours in total) as well as relocation. Importantly however, there was no difference in demographic features of those participants who remained in the study and those lost to follow-up. This study utilised data from a longitudinal cohort study investigating the relationship between biomechanical factors and joint structure. As such, the study was not specifically set up to address the research questions in this thesis and an a priori
sample size calculation was not performed. The sample size was relatively small, particularly for the combined ACLR group and the control group. Clearly, this reduces the statistical power of the study and increases the likelihood of a Type II error. Indeed, a sample size of 32 participants provides an effect size of 0.5 to detect cartilage volume change within each group with 80% power and an alpha level of 0.05. With 25 participants, the power is 70%, and with 9 participants, the power decreased to 32%. However, large numbers of statistical tests were performed and this can increase the risk of a Type I error. Given the exploratory nature of the study, no adjustments were made to the alpha level. Future studies with larger sample sizes and longer follow-up are required to confirm the results of this study.

5.5 Conclusion

Cartilage volume increases occurred at the medial tibia, lateral tibia and patella from 2 to 4 years following ACLR, with the isolated ACLR group showing greater increases compared with the combined meniscal pathology group and with healthy controls. Cartilage defects in the patella of isolated ACLR participants and BMLs in the medial tibia of participants in the combined ACLR group exhibited regression over 2-year period. A greater proportion of ACLR participants in the isolated and combined groups exhibited BML regression in the lateral tibia compared with controls. Baseline cartilage defect and BMLs scores were associated with cartilage volume change in ACLR participants. Collectively, the results of this study indicate that changes in ACLR knee joint structure over a two-year follow-up period are dependent upon a) the structural feature and compartment of interest and, b) whether concomitant meniscal pathology is present.
Chapter 6: Cartilage quantitative T2 relaxation time from 2.5 to 4.5 years following anterior cruciate ligament reconstruction

6.1 Introduction

Anterior cruciate ligament rupture is a common knee injury, and surgical reconstruction (ACLR) is frequently performed to restore knee stability and function (Chalmers et al., 2014; Fu et al., 2000). However, ACL rupture and concomitant meniscal injury are strongly associated with an increased risk of knee OA even after ACLR (Ajuied et al., 2014; Lohmander et al., 2007). The ACLR knee constitutes an ideal model for elucidating the pathogenesis of OA and the benefits of disease-slowing interventions given that those who have undergone ACLR have accelerated degeneration of cartilage and subchondral bone (Eckstein et al., 2015; Hunter et al., 2014).

Early-stage cartilage degeneration is characterised by a loss of proteoglycans, collagen disorganization and increasing water content (Dijkgraaf et al., 1995). Cartilage biochemical and compositional changes occur with altered mechanical loading that, in turn, exposes the cartilage to further morphological degradation (Binks et al., 2013). Advanced compositional MRI techniques such as T2 relaxation time, T1rho relaxation time and delayed gadolinium-enhanced MRI of cartilage (dGEMRIC), can quantify cartilage biochemical changes and detect early degeneration non-invasively (Baum et al., 2013). T2 relaxation time, or T2 value, reflects the water content and collagen fibril orientation; thus, an elevated T2 value suggests increased water permeability as well as collagen damage early in the degenerative process (Dunn et al., 2004; Lusse et al., 2000; Nissi et al., 2006).

In previous cross-sectional studies, ACL injured individuals exhibited higher T2 cartilage values in the posterior aspect of the lateral tibia compared with healthy
controls at 1 month post-injury and at 1 year following subsequent ACLR (Su et al., 2013). The higher cartilage T2 values in the lateral tibia are regarded as an ACL injury ‘footprint’ - just like post-traumatic bone marrow lesions (BMLs) (Li et al., 2011; Sanders et al., 2000; Su et al., 2013). However, lateral tibia T2 values at 2 years following ACLR were not different between ACLR participants and healthy controls (Su et al., 2013). Compared with healthy controls, higher T2 values were also found in the medial femoral condyle at 6 months and 2 years post-surgery (Su et al., 2013; Van Ginckel et al., 2013).

The time course in the change of T2 values following ACLR is currently unclear due to the paucity of longitudinal investigations. In one study, 40 patients were followed for 11 years following ACLR (Potter et al., 2012). While the authors reported progressively higher T2 values in the lateral compartment compared with those at year one, no quantitative data were provided. In an earlier study incorporating quantitative analysis of ACLR patients, T2 values did not change significantly over the first year; however, there was a trend towards a decrease in the lateral tibia and an increase in the medial femoral condyle (Li et al., 2011). Similarly, Su et al. (2013) reported that T2 values in the lateral tibia decreased over the first two years post-ACLR (Su et al. 2013). A decreasing T2 value in the cartilage of the lateral tibia might suggest a partial recovery after the trauma associated with ACL rupture. However, the trend toward increasing T2 values at the medial femoral condyle indicates differential, region-specific cartilage responses. Investigation of T2 value changes at different cartilage regions and spanning more extended time frames from ACL injury is needed to provide further insight into early cartilage structural alterations in this population.

Considering that the structural and biomechanical properties of cartilage are different through the depth of the tissue, laminar analysis of T2 values has been adopted to improve the understanding of degenerative process of articular cartilage (Buckwalter, 1998; Eyre, 2002; Kumar et al., 2014; Li et al., 2011). Cartilage T2 values were
calculated separately for the superficial (articular) and deep (subchondral bone) layer in individuals with ACLR (Li et al., 2011). Laminar analysis suggested that there are degenerative changes in the superficial layer similar to the full-thickness analysis at medial femoral condyle in ACLR participants (Li et al., 2011; Su et al., 2013). Importantly, although lateral tibia T2 values in the deep layer did not differ between ACLR patients and healthy controls at baseline, higher T2 values were observed in ACLR patients at one and two years follow-up (Su et al., 2013). In another study including older adults with and without isolated meniscal tear, higher T2 values were also observed in the deep layer of cartilage across all compartments together with subchondral bone degradation (Kumar et al., 2013). Therefore, laminar analysis of T2 values may help further elucidate the degenerative process in the OA. Current literature has only documented cartilage T2 values between ACLR participants and healthy controls up to two years following surgery (Su et al., 2013), and the cartilage T2 values thereafter remain unclear.

### 6.1.1 Study aims and hypotheses

This was an exploratory study investigating cartilage health following ACLR using T2 mapping. The primary aim was to compare the cartilage T2 values (full-thickness and laminar) between ACLR participants at 2.5 years post-surgery and healthy control participants. It was hypothesised that ACLR individuals will exhibit higher knee cartilage T2 values compared with controls at the medial femoral condyle (full-thickness) and lateral tibia (deep layer) ($H_1$).

The secondary aim was to examine the change in cartilage T2 values over 2 years in ACLR participants in the medial femoral condyle and lateral tibia. Based on the findings of the limited literature currently available, it was hypothesised that cartilage T2 values will increase in the medial femoral condyle and decrease in the lateral tibia of ACLR participants over 2 years ($H_2$). Change of T2 values were expected to be similar in the full-thickness and laminar analysis.
6.2 Methods

6.2.1 Participants

The participants in this study were a subset of the cohort described in Chapter 2 (section 2.1). All 34 participants in Melbourne with an isolated ACLR without meniscal pathology underwent an additional scan at baseline. However, technical scanning issues for 6 participants precluded analysis of their data. Of the remaining 28 participants, 16 returned for follow-up T2 scans after 2 years. Nine healthy participants with comparable physical activity levels were recruited at baseline as the reference group but due to funding constraints, did not undergo repeat testing at 2 years. The recruitment procedures (Chapter 2, Section 2.2.1) and eligibility criteria (Chapter 2, section 2.2.2) have been described previously.

6.2.2 Surgery and rehabilitation procedure

As the surgery and rehabilitation procedure for the ACLR group have been described in detail in Chapter 3 (section 3.2.3), only a brief summary is included. All ACLR were performed arthroscopically using the hamstring tendon autografts. None of the participants had combined meniscal pathology.

6.2.3 Anthropometric, physical activity and MRI assessments

Height, weight and BMI

Mass was measured to the nearest 0.1 kg using electronic scales. Height was measured to the nearest 0.1 cm using a stadiometer. Weight and height were used to calculate body mass index (BMI; kg/m²).

Sport activity level

The sports activity rating scale from the Cincinnati knee rating system was used to assess the activity level of the participants by considering both the frequency of play
and the type of sports (Noyes et al., 1989). Higher scores indicate higher level of sports participation (0-100).

**MRI assessment**

Quantitative T2 mapping was performed using one 3-T MRI unit in Melbourne (Siemens MagnetomVerio, Erlangen, Germany). T2 mapping used a sagittal multi-echo spin echo sequence, implementing a slice thickness of 3 mm; echo time/repetition time of 13.8, 27.6, 41.4, 55.2, and 69.0/1200 milliseconds; Flip Angle 180 degree; field of view 330 mm; matrix of 256×256 pixels (in-plane resolution: 0.42×0.42); and acquisition time of 8 min 16 sec. As described in chapter 2 (section 3.5.5), the MRI scan included morphology sequences (proton-density and T1-weighted 3D gradient recall acquisition) and T2 mapping sequence. The T2 mapping sequence was performed after the morphology sequence following 20 minutes in the supine position.

T2 values were derived from the Epworth Hospital in Melbourne using vendor-supplied software (Siemens syngoMapIt, Erlangen, Germany). Cartilage was segmented by manually tracing the boundary in the medial tibia, medial femoral condyle, lateral tibia, lateral femoral condyle, trochlear and patella using Osirix (University of Geneva, Geneva, Switzerland) (Figure 6.1). After delineating the full-thickness cartilage, the entire layer was further divided into two equally spaced layers (i.e., superficial layer and deep layer) using an in-house program developed in MatLab (The Mathworks, Inc. Mass., USA) (Figure 6.2). Full-thickness and laminar T2 values were calculated using the same program. T2 values were averaged from the middle five slices of cartilage in the tibiofemoral compartment (i.e., medial tibia, medial femoral condyle, lateral tibia and lateral femoral condyle) (Li et al., 2013) and 10 slices in the patella. T2 values in the trochlear groove were measured in central 3 slices using axial plane images transformed by the software. The cartilage segmentation for T2 measurement takes approximately 45 minutes in each knee joint.
Pixels with relaxation time values greater than 100 msec on T2 were removed from the data to reduce artefacts caused by partial volume effects. A trained observer (XW) performed all T2 segmentation. The intra-rater reliability was determined from 10 participants on two separate occasions 7 days apart. ICCs for medial tibia, medial femoral condyle, lateral tibia, lateral femoral condyle, trochlear and patella were 0.95, 0.98, 0.96, 0.94, 0.99 and 0.98, respectively.

Figure 6.1 The segmentation of cartilage regions in the medial tibia (A), medial femoral condyle (B), lateral tibia (C), lateral femoral condyle (D), trochlear (E) and patella (F).
6.2.4 Statistical analysis

The assumption of normality was examined using Shapiro-wilk test. Means and standard deviations were presented for continuous variables with normal distribution, while median and interquartile range presented for continuous variables that were not normally distributed. For descriptive variables, Chi square, Mann-Whitney U or independent samples t-tests were used to compare between groups, where appropriate. Independent samples t-test was performed to compare the baseline T2 values without adjustment, while analysis of covariance (ANCOVA) was used to compare differences after adjusting for age, gender and BMI. Paired samples t-test was used to examine the longitudinal change of cartilage T2 value in ACLR participants. All statistical analyses were performed using SPSS package (version 22.0, SPSS, Chicago, IBM) with significance accepted at P < 0.05.
6.3 Results

6.3.1 Comparison of baseline cartilage T2 value between ACLR and controls

Participants

The isolated ACLR participants in this study (n=28) were a subgroup of the isolated ACLR participants (n=34). As shown in Table 6.1, there were no significant differences in demographic characteristics between ACLR participants included in this study and those in Chapter 4.

Table 6.1 Demographic characteristics comparing the ACLR participants with all ACLR participants in Melbourne

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>ACLR for T2 analysis (n = 28)</th>
<th>All ACLR (n = 34)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>29.8 (± 6.3)</td>
<td>29.7 (± 6.1)</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>17 (61%)</td>
<td>21 (62%)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.2 (± 2.5)</td>
<td>24.0 (± 2.7)</td>
</tr>
<tr>
<td>Sports activity level</td>
<td>85 (80, 95)</td>
<td>85 (80, 95)</td>
</tr>
<tr>
<td>Time from surgery to baseline assessment (yr)</td>
<td>2.4 (± 0.5)</td>
<td>2.4 (± 0.5)</td>
</tr>
</tbody>
</table>

Parametric data presented as mean (± standard deviation), and sports activity level presented as median (interquartile range). BMI=body mass index. Sport activity level ranges from 0 to100, and higher scores indicate higher level of sports participation.

A comparison of the demographics of participants at baseline in the isolated ACLR group and control group is shown in Table 5.2. Participants in the ACLR group had a higher BMI than those in the control group (P = 0.01). No significant differences were found for the other variables.
Table 6.2 Demographics comparing the isolated ACLR and control groups

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>ACLR (n = 28)</th>
<th>Controls (n = 9)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>29.8 (± 6.3)</td>
<td>26.0 (± 4.7)</td>
<td>0.10</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>17 (61%)</td>
<td>4 (44%)</td>
<td>0.46</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.2 (± 2.5)</td>
<td>21.3 (± 3.1)</td>
<td>0.01*</td>
</tr>
<tr>
<td>Sports activity level</td>
<td>85 (80, 95)</td>
<td>95 (87, 100)</td>
<td>0.08</td>
</tr>
<tr>
<td>Time from surgery to baseline assessment (yr)</td>
<td>2.4 (± 0.5)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Table 6.2 Demographics comparing the isolated ACLR and control groups

BMI=body mass index. N/A=not applicable. Parametric data were presented as mean (± standard deviation), and sports activity level was presented as median (interquartile range). Sport activity level ranges from 0 to 100 with higher scores indicating higher level of sports participation.

* Significant difference (P< 0.05).

Comparison of baseline cartilage T2 value between ACLR and control groups

Cartilage T2 values at baseline in ACLR and control groups, together with unadjusted and adjusted between-group differences are shown in Table 6.3. Without adjustment, the ACLR group showed a significantly higher T2 value in the full-thickness of the medial femoral condyle (P = 0.04) and the deep layer of the medial tibia (P = 0.03). After adjustment for age, gender and BMI, the ACLR group exhibited higher T2 values in the deep layer of the medial femoral condyle (P = 0.03, Table 6.3). The results were also similar when including physical activity level as an additional covariate given a trend toward lower activity levels in the ACLR group. No significant differences were found at other sites.
Table 6.3 Mean + standard deviation of cartilage T2 values in ACLR group and control groups together with mean (95% confidence intervals) between-group differences

<table>
<thead>
<tr>
<th></th>
<th>Full-thickness T2 (ms)</th>
<th>Superficial layer T2 (ms)</th>
<th>Deep layer T2 (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ACLR</td>
<td>Controls</td>
<td>Unadjusted difference (95% CI)</td>
</tr>
<tr>
<td>Medial tibia</td>
<td>32.4 ± 4.2</td>
<td>29.3 ± 4.8</td>
<td>3.1 (-0.3, 6.5)</td>
</tr>
<tr>
<td>Medial femoral condyle</td>
<td>42.0 ± 3.8</td>
<td>38.9 ± 3.9</td>
<td>3.1 (0.2, 6.0)*</td>
</tr>
<tr>
<td>Lateral tibia</td>
<td>28.8 ± 4.0</td>
<td>29.2 ± 4.2</td>
<td>-0.3 (-3.5, 2.8)</td>
</tr>
<tr>
<td>Lateral femoral condyle</td>
<td>42.7 ± 4.3</td>
<td>40.8 ± 3.5</td>
<td>1.9 (-1.3, 5.1)</td>
</tr>
<tr>
<td>Trochlear</td>
<td>46.2 ± 3.8</td>
<td>47.8 ± 6.1</td>
<td>1.6 (-2.8, 6.0)</td>
</tr>
<tr>
<td>Patella</td>
<td>36.1 ± 4.9</td>
<td>37.6 ± 4.1</td>
<td>-1.5 (-5.2, 2.2)</td>
</tr>
</tbody>
</table>

T2 values are presented as mean (± standard deviation). 95% CI=95% confidence interval. A positive difference means T2 values were higher in ACLR group.* Significant difference between the two groups ($P<0.05$). # Adjusting for age, gender and BMI.
6.3.2 Longitudinal changes in T2 values in ACLR participants over 2 years

Participants

The demographic characteristics of the 16 ACLR participants (57%) who returned for follow-up assessment and the 12 ACLR participants who were lost to follow-up are shown in Table 6.4. Reasons for loss to follow-up included: 2 could not be contacted, 4 were too busy, 3 had an additional ACL or meniscal injury and 3 had MRI contraindications including pregnancy (n=2) and wearing an intrauterine device (n=1). There was no significant difference in demographics between ACLR participants who completed the longitudinal study and those who were lost to follow-up (Table 6.4).

Table 6.4 Comparison between ACLR participants who returned for follow-up and those who withdrew

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Completed follow-up (n = 16)</th>
<th>Lost to follow-up (n = 12)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>30.5 (± 6.9)</td>
<td>29.0 (± 5.7)</td>
<td>0.55</td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>10 (63%)</td>
<td>7 (47%)</td>
<td>0.14</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.9 (± 3.1)</td>
<td>24.4 (± 1.8)</td>
<td>0.63</td>
</tr>
<tr>
<td>Sports activity level</td>
<td>85 (80, 95)</td>
<td>90 (76, 95)</td>
<td>0.94</td>
</tr>
<tr>
<td>Time from surgery to baseline assessment (yr)</td>
<td>2.5 (± 0.4)</td>
<td>2.4 (± 0.5)</td>
<td>0.79</td>
</tr>
<tr>
<td>Time between baseline and follow-up (yr)</td>
<td>2.1 (± 0.3)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

BMI=body mass index. N/A=not applicable. Parametric data were presented as mean (± standard deviation), and sports activity level was presented as median (interquartile range).

Longitudinal T2 value changes

The 2-year change in cartilage T2 values are shown in Table 6.5. ACLR participants exhibited a significant decrease in T2 values in the deep layer of the lateral tibia (P = 0.04, mean difference 1.4ms [6.9%], 95% CI 0.04, 2.8ms). No significant changes in T2 values were identified at the other sites.
Table 6.5 Two-year change of T2 value in ACLR participants from baseline to follow-up using paired-t test

<table>
<thead>
<tr>
<th>Location</th>
<th>Full-thickness T2 (ms)</th>
<th>Superficial layer T2 (ms)</th>
<th>Deep layer T2 (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BL</td>
<td>FU</td>
<td>Mean change (95% CI)</td>
</tr>
<tr>
<td>Medial tibia</td>
<td>32.2</td>
<td>32.0</td>
<td>-0.2 (-1.6, 1.1)</td>
</tr>
<tr>
<td></td>
<td>4.8</td>
<td>4.8</td>
<td>±</td>
</tr>
<tr>
<td>Medial femoral condyle</td>
<td>41.1</td>
<td>41.1</td>
<td>0.01 (-1.3, 1.5)</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td>3.7</td>
<td>±</td>
</tr>
<tr>
<td>Lateral tibia</td>
<td>29.0</td>
<td>27.7</td>
<td>-1.3 (-2.9, 0.4)</td>
</tr>
<tr>
<td></td>
<td>3.2</td>
<td>2.3</td>
<td>±</td>
</tr>
<tr>
<td>Lateral femoral condyle</td>
<td>42.4</td>
<td>42.3</td>
<td>-0.1 (-1.2, 1.4)</td>
</tr>
<tr>
<td></td>
<td>4.5</td>
<td>4.2</td>
<td>±</td>
</tr>
<tr>
<td>Trochlear</td>
<td>46.0</td>
<td>45.3</td>
<td>-0.7 (-3.2, 1.8)</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>5.2</td>
<td>±</td>
</tr>
<tr>
<td>Patella</td>
<td>36.2</td>
<td>36.6</td>
<td>0.4 (-1.5, 2.2)</td>
</tr>
<tr>
<td></td>
<td>5.4</td>
<td>3.9</td>
<td>±</td>
</tr>
</tbody>
</table>

T2 values presented as mean (± standard deviation). 95% CI=95% confidence interval. Change of T2 = follow-up T2 – baseline T2, thus negative values represent a decrease in T2 while positive values represent an increase. BL=Baseline; FU=Follow-up. * Significant change (P< 0.05).
6.4 Discussion:

The most important finding of the present study was that ACLR participants showed higher T2 values in the medial femoral condyle at 2.5 years following surgery compared to controls. At the 2-year follow-up, ACLR participants showed a significant reduction in T2 value in the deep layer of lateral tibia. No changes in follow-up T2 values were found at other sites.

Comparatively higher cartilage T2 value in the medial femoral condyle of ACLR participants at baseline is suggestive of degenerative changes, namely damaged collagen network and associated increased mobility of water. Furthermore, laminar analysis revealed higher T2 values in the deep layer of medial femoral condyle, suggesting that cartilage degeneration is isolated to the deep layer. The ACLR participants also exhibited a trend towards higher T2 values in the deep layer of medial tibia prior to adjustment of confounders; however, values were not significantly different after adjustment. Previous studies have also reported higher full-thickness and superficial layer T2 values in the medial femoral condyle of ACLR patients compared with healthy controls (Su et al., 2013; Van Ginckel et al., 2013). Moreover, compositional MRI studies using T1rho value and delayed gadolinium-enhanced MRI of cartilage value (dGEMRIC) have also noted cartilage degeneration (i.e., proteoglycan loss) in the medial femoral condyle from 3 weeks to 2 years following ACLR (Hirose et al., 2013; Li et al., 2011; Neuman et al., 2011; Su et al., 2013; Tiderius et al., 2005). The present study found higher T2 values not only in the full-thickness cartilage at the medial femoral condyle but also in the deep layer. The interaction between cartilage and underlying subchondral bone is thought to contribute to degenerative changes associated with OA (Burr et al., 2012; Kumar et al., 2013). Flow of synovial fluid in healthy cartilage is restricted by the deep calcified cartilage layer; however, fluid permeability in diseased OA cartilage increases as a result of angiogenesis (i.e., invasion of blood vessels) in the calcified cartilage as well as progression of cartilage fissures at the cartilage surface (Lories et al., 2011; Pesesse et al., 2013). Higher T2 values in the deep cartilage layer - probably caused by increased hydraulic permeability in the cartilage-subchondral bone unit - have been associated with cartilage degeneration (Hwang et al., 2008; Setton et al., 1995). One study found that patients with medial meniscal tear exhibited higher T2 values together with subchondral bone resorptive change compared those without meniscal tear (Kumar et al., 2013).
Interestingly, there was no difference in baseline T2 values in the superficial layer of medial femoral condyle between ACLR and control participants. This finding may be related to the control group’s participation in high levels of physical activity given that repetitive loading via strenuous exercise has been linked with higher cartilage T2 values in asymptomatic, middle-aged individuals (Hovis et al., 2011; Lin et al., 2013). Specifically, it is possible that repetitive loading of healthy cartilage over an extended period contributes to disruption of collagen and, in turn, elevated cartilage T2 values (Lin et al., 2013). Taken together, higher full-thickness T2 values at the medial femoral condyle of ACLR participants are probably related to T2 changes in the deep cartilage layers.

In contrast to the H1, there was no difference in the T2 values of the deep layer of lateral tibia between ACLR participants and controls at baseline. This finding is in contrast to a previous study that reported higher T2 values in the deep layer of lateral tibia at 2 years following ACLR (Su et al., 2013). Since higher T2 values are thought to represent cartilage degeneration (Baum et al., 2013; Guermazi et al., 2015), normalisation of lateral tibia T2 values in the present may indicate partial recovery of deep layer cartilage microstructure and composition. Similarly, two studies have reported that elevated cartilage T2 values at one-month post ACL injury progressively decrease over the subsequent 2 years at this site (Li et al., 2011; Su et al., 2013). Indeed, it is important to note that changes in cartilage composition are a dynamic process, and increased synthesis of collagen and proteoglycan is part of the cartilage repair process, especially in the early stages of OA (Brandt et al., 1991; Eyre et al., 1980; Sandy et al., 1984). However, it is also equally important to emphasis that newly synthesised cartilage is different from healthy cartilage in that new cartilage exhibits poorer mechanical properties and, as such, is more susceptible to the degeneration in the longer-term (Buckwalter et al., 2004; Dijkgraaf et al., 1995; Eyre et al., 1980).

With respect to longitudinal change in ACLR participants, the current study found a decrease in T2 value in the deep layer of lateral tibia over the 2-year follow-up (in support of H2). As mentioned, partial restoration of cartilage composition has been found in lateral tibia in the first 2 years following ACLR (Su et al., 2013), and the findings of the current study tend to suggest that cartilage regeneration in the deep layer of lateral tibia extends up to 4.5 years following surgery. Whilst there was no significant change in T2 values in the medial femoral condyle (in contrast to H2), a trend opposite that observed at lateral tibia.
was apparent; that is, laminar analysis showed increased T2 values in the superficial cartilage layer and a decrease in the deep cartilage layer. This trend may be the result of altered loading of the lateral compartment of ACLR participants, given that several studies have reported T2 values changes under different knee joint loading (Kumar et al., 2014; Nishii et al., 2008; Souza et al., 2012). Specifically, Souza et al. (2014) found that T2 values in the medial femoral condyle of both healthy and OA individuals decreased in the superficial layer and increased in the deep layer following controlled knee joint loading in a custom-made mechanical loading system during an MRI scan. By contrast however, biomechanical analyses of ACLR participants included in the present study demonstrated pronounced under-loading in the medial tibiofemoral compartment during walking, running and cutting (data not shown; Saxby et al. in review). Hence, over the two-year follow-up period, constant under-loading of the medial femoral condyle in ACLR participants may have been a contributing factor to the reversal in superficial and deep T2 cartilage values. Clearly, this assumption requires future investigation.

Longitudinal changes in T2 cartilage values were not significant at other sites, except the lateral tibia and this was probably due to study duration and/or ceiling effects. Specifically, a recent study investigated cartilage thickness changes over the first 5 years following ACLR and thickness changes were greater over the first 2 years than over the subsequent 3 years, suggesting that major perturbations in cartilage homeostasis occur in the first 2 years following ACLR (Eckstein et al., 2015). Similarly, the magnitude of T2 cartilage change across the two-year follow-up may be less pronounced compared to early changes during the first 2.5 years post-ACLR. With respect to ceiling effects, T2 mapping is a widely available MRI technique and has been used in multi-center observational study Osteoarthritis Initiative; however, it has been suggested that T2 mapping data may be subject to ceiling effects(Jungmann et al., 2013; Kumar et al., 2014).Indeed, the dynamic range of T2 mapping analyses for detecting small macromolecular changes appear to be limited (Li et al., 2011; Regatte et al., 2006; Su et al., 2013).This notion was supported by Jungmann et al. (2013) who reported that older, healthy individuals with higher baseline T2 values were associated with smaller T2 increase over a 2-year follow-up period(Jungmann et al., 2013).

There are several strengths in the present study. Firstly, this study is the first to compare T2 cartilage values between ACLR participants and healthy controls at 2.5 years following
surgery and to explore changes over the subsequent 2 years. A recent study by Bae et al. (2015) reported higher medial femoral condyle T2 cartilage values in the injured knee versus contralateral knee of ACLR patients at 3 years following surgery (Bae et al., 2015). However, it is important to note that within-participant study designs are limited in that the contralateral knee of ACLR patients is subject to compensatory biomechanical changes that may result in knee contact forces that are augmented compared to matched, healthy control knees (Zabala et al., 2013). Secondly, recruiting ACLR participants without concomitant meniscal pathology increased the homogeneity of the cohort. Combined meniscal pathology increases the risk of cartilage degeneration compared with isolated ACLR (Oiestad et al., 2009), and T2 values in patients with combined ACLR and meniscal pathology have been reported to be higher than in those with isolated ACLR (Li et al., 2015).

This study also has several limitations. First, the sample size was small, and this reduced the statistical power of the study. Second, only 16 of the 28 ACLR participants (57%) returned for follow-up testing. That said, there were no differences in demographics between ACLR participants who returned for follow-up and those who did not. Third, control participants were only assessed at baseline and, as such, it is difficult to determine whether the longitudinal changes in T2 cartilage values are the result of ACL injury and subsequent surgery or simply natural, time-related changes. However, results from an unpublished study (Su et al., 2013) indicate no T2 cartilage changes over a 2-year period in healthy individuals (Su et al., 2013). Fourth, although sub-regional analysis of T2 value was used in several studies (Li et al., 2011; Su et al., 2013), it was not performed in this study. T2 value measurement in the whole cartilage plate may be less sensitive to subtle changes than in sub-regions; however, the partial volume effect of the MR image could be reduced by including more pixels, which increased the reliability of the outcome.

6.5 Conclusion

ACLR individuals exhibited higher T2 value, suggestive of cartilage degeneration in the medial femoral condyle compared with healthy controls at 2.5 years following surgery. The decreased T2 value in the deep layer of lateral tibia from 2.5 to 4.5 years following ACLR suggests a partial restoration of cartilage composition.
7.1 Thesis summary

The analyses conducted for this thesis utilised data from a longitudinal cohort study assessing joint morphology and cartilage composition in young adults following ACLR with or without combined meniscal pathology, and control participants. This thesis aimed to elucidate a greater understanding of the natural history and pathogenesis of post-traumatic OA from the imaging perspective. Joint morphology (i.e., cartilage volume, cartilage defects and BMLs) and cartilage compositional status (i.e., T2 values) were assessed using MRI as this technique is sensitive to the early changes of joint degeneration.

Chapter 4 reported the findings comparing joint morphology in the three groups at baseline (2.5 years following ACLR). Specifically, ACLR patients exhibited worse features (i.e., smaller cartilage volume, higher prevalence of cartilage defects and BMLs) compared with healthy controls while the combined ACLR group showed a higher prevalence of cartilage defects than the isolated ACLR group. Cartilage defects and BMLs in ACLR patients are often referred to as post-traumatic footprints indicating the site of damage at the time of injury. Cartilage defects presented at all cartilage sites except for the medial tibia, while a high prevalence of BMLs was detected in the lateral tibia. In addition, BMLs in the medial tibia were more prevalent in the combined ACLR group compared with healthy controls.

Chapter 5 reported the natural history of joint morphological changes over a 2-year follow-up period. An increase in cartilage volume was found at all sites in both groups of ACLR patients – a finding that accords with the notion that cartilage swelling or hypertrophy is indicative of early cartilage degeneration. Cartilage defects remained unchanged in most ACLR patients, suggesting that cartilage defects either do not
change from 2.5 to 4.5 years following ACLR or that the extent of any changes are not
detectable by MRI. However, regression of patella cartilage defects occurred in the
isolated ACLR participants. This suggests that the natural course of cartilage defects is
not a unidirectional process of progression but that hypertrophic repair can occur in
some regions. BML scores exhibited a significant improvement in the combined
ACLR group in the medial tibia where a high prevalence was found at baseline.
Whilst the mechanism of improvement is unknown, altered joint compartmental
loading may play a role.

Chapter 5 also reported comparisons of joint changes between the three groups to help
understand the pathogenesis of post-traumatic OA. The isolated ACLR group showed
a greater annual increase in cartilage volume compared with both the control group and
the combined ACLR group. This finding supported the notion that an increase in
cartilage volume is an early form of degeneration, while cartilage volume loss occurs in
more advanced stages of degeneration. Furthermore, the combined ACLR group also
showed a greater increase in cartilage volume compared with healthy controls,
although it did not reach statistical significance. Thus, early degeneration (cartilage
increase) and late degeneration (cartilage loss) could occur at the same time within a
joint compartment, and the final magnitude of cartilage volume change in the
compartment depends upon the balance of cartilage increase and cartilage loss at
different sites. Changes in cartilage defects did not differ between three groups,
probably because most cartilage defect scores did not change over the 2 years.
Interestingly, a greater number of participants in the isolated ACLR group showed
regression of BMLs in the lateral tibia compared with the control group, indicating that
BMLs in the lateral tibia continue to resolve from 2.5 to 4.5 years following ACLR.

Chapter 5 also examined the association between baseline cartilage defects and BML
scores and subsequent cartilage volume change over 2 years in ACLR participants in
order to demonstrate the role of cartilage and subchondral bone pathology in the
pathophysiology of post-traumatic OA. Higher baseline cartilage defect scores were associated with greater cartilage volume increase in the lateral tibia and patella, while the association just failed to reach statistical significance in the medial tibia. This suggests that cartilage injury, as reflected by cartilage defects, may contribute to the subsequent early cartilage degenerative changes, and this is a universal finding at different cartilage sites. By contrast, greater size of baseline BMLs in the medial tibia were associated with cartilage volume loss, suggesting that BMLs in the medial tibia may impede the hypertrophic repair response of the overlying cartilage. Given that the association was restricted to severe BMLs in the medial tibia only, subchondral bone pathology, in combination with other factors including joint loading and/or local inflammation, may lead to cartilage degeneration.

Chapter 6 reported the cartilage compositional status using MRI biomarker T2 values in the patients with isolated ACLR and controls. ACLR individuals exhibited higher T2 value in the medial femoral condyle at baseline (2.5 years following surgery) compared with healthy controls – a finding suggestive of cartilage degeneration at this site. The ACLR participants demonstrated decreased T2 value in the deep layer of lateral tibia from 2.5 to 4.5 years, suggesting a partial restoration of cartilage composition.

In summary, the results of this thesis make a substantive contribution to the literature as they demonstrate the changes in joint morphology and cartilage composition in young individuals following ACLR. This thesis also provides evidence that the development of post-traumatic OA appears to be associated with pathology in both the cartilage and subchondral bone. The key points and findings of the thesis was summarised in Table 7.1.
Table 7.1 Conclusion table of the thesis

<table>
<thead>
<tr>
<th>Number of chapters</th>
<th>Key points and findings</th>
</tr>
</thead>
</table>
| Chapter 4          | • At 2-3 years following ACLR, cartilage exhibited more defects and smaller volume compared with that of the uninjured healthy individuals.  
• ACLR with concomitant meniscal injury had a more pronounced detrimental effect on cartilage morphology than isolated ACLR.  
• Current findings suggest that ACLR patients with associated meniscal pathology are subject to worse cartilage features than isolated ACLR patients, leading to a greater risk of OA in the long term. |
| Chapter 5          | • An increase in cartilage volume was found at all sites in both groups of ACLR patients – a finding that accords with the notion that cartilage swelling or hypertrophy is indicative of early cartilage degeneration.  
• The combined ACLR group exhibited a more advanced level of cartilage degeneration than the isolated ACLR group.  
• The natural course of cartilage defects is not a unidirectional process of progression but that hypertrophic repair can occur in some regions.  
• Baseline cartilage defects predispose the cartilage to degenerative changes, suggesting that cartilage injury contributes to the subsequent early degeneration.  
• The presence of baseline large size BMLs in the medial tibia is a risk factor contributing to subsequent degenerative change in cartilage.  
• Joint structure changes following ACLR over a two-year follow-up period are dependent upon a) the structural feature and compartment of interest and, b) whether concomitant meniscal pathology is present.  
• The development of post-traumatic OA appears to be associated with pathology in both the cartilage and subchondral bone. |
| Chapter 6          | • ACLR individuals exhibited higher T2 value compared with healthy controls, suggestive of cartilage degeneration in the medial femoral condyle at 2.5 years following surgery.  
• ACLR individuals exhibited decreased T2 value in the deep layer of lateral tibia over 2-year follow-up, which suggests a partial restoration of cartilage composition. |
7.2 Clinical implications

The main clinical implication of the research within this thesis is that the pathologic changes in the cartilage and subchondral bone are associated with subsequent cartilage degeneration and the development of post-traumatic knee OA. Thus, the treatment of these pathologic changes including cartilage defects and BMLs may be necessary to reduce the risk of early-onset post-traumatic OA in young people following ACLR.

Findings from this thesis (Chapter 5) demonstrated that even mild damage to the cartilage results in degenerative change; thus, clinicians should take cartilage defects into consideration when counselling ACLR patients about the long-term risks of cartilage degeneration. Specifically, all baseline cartilage defects in the current ACLR cohort were ICRS grade 1-2, and grade 1-2 cartilage defects frequently referred to as ‘no chondral damage’ or ‘low-risk for future OA’ (Ichiba et al., 2009; Shelbourne et al., 2003; Wasilko et al., 2015). The current study provided evidence that the presence of baseline cartilage defects, even they are mild damage, are associated with early cartilage degeneration in the subsequent 2 years. Therefore, treating cartilage defects early may be important to reduce the risk of early OA. In current clinical practice, focal cartilage defects are commonly managed by microfracture, autologous chondrocyte implantation (ACI), osteochondral autologous transplantation (OAT) and mesenchymal stem cell therapies; however, the efficacy and long-term consequence of these treatments still needs to be determined (J. A. Anderson et al., 2014; Bekkers et al., 2009).

Apart from cartilage repair techniques, the timing of ACLR itself could prevent further damage to the surrounding joint structures. Indeed, previous studies recommended that ACLR is performed within the first year following the injury to minimise the secondary damage to the meniscus and cartilage (Church et al., 2005; Kennedy et al., 2010; Michalitsis et al., 2015). Consistent with this, participants in the
current study underwent the ACLR within six months post-injury, and they exhibited little change in cartilage defects. Thus, the clinical implication is that the treatment of cartilage defects may be necessary to prevent advanced degeneration, and acute ACLR could be helpful in the prevention of secondary cartilage defects. However, this requires further confirmatory research.

This thesis demonstrated that large BMLs in the medial tibia are a precursor to subsequent cartilage loss. As such, it may be that rehabilitation following ACLR should be less aggressive when large BMLs are identified. Today, early weight-bearing and accelerated rehabilitation are advocated in most patients following ACLR with the aim of reducing complications such as loss of motion and residual weakness (Micheo et al., 2010; Nakamae et al., 2006). Although previous studies demonstrated better improvement in thigh muscle strength and morphology using the accelerated rehabilitation compared with non-accelerated rehabilitation (Beynnon et al., 2005; Gerber et al., 2007), the presence of large and severe BML is clinically concerning, given the altered mechanical properties in cartilage and subchondral bone, disrupted biosynthesis and cell death (Johnson et al., 1998). The association between BML size and subsequent cartilage degeneration was noted in the lateral compartment in the first three years but not after; however, the long-term impact of BMLs in the medial compartment is not clear (Deangelis et al., 2010; Potter et al., 2012; Roemer et al., 2009).

The data presented in Chapter 5 suggest that large and severe BMLs in the medial tibia are of clinical relevance in ACLR patients. It may be reasonable to delay full weight-bearing and unload the knee of ACLR patients to prevent further cartilage loss when clinicians find large and severe BMLs in the medial tibia. Although the effects of unloading for treating BMLs is uncertain at this point, previous studies have demonstrated that augmented loading increases the presence of BMLs in the medial compartment and unloading the knee has a beneficial effect on the joint space in the
OA population (Beckwee et al., 2015; Bennell et al., 2010; Wiegant et al., 2013). Large and severe BMLs were associated with cartilage loss in the medial tibia only; thus, both the location and size of BMLs may need to be considered for predicting long-term outcome.

7.3 Strengths of the study

The strengths of the study have already been discussed in corresponding chapters, so only a brief overview will be presented in this section.

The first strength of this research is the strict eligibility criteria and the longitudinal study design. The strict eligibility criteria resulted in the inclusion of a homogeneous ACLR cohort, which is important to maximise the internal validity of the findings. These patients following ACLR are suitable to observe the early degeneration in the knee joint, and characteristics of participants in the current study are comparable to previous studies such as the KANON trial (Eckstein et al., 2015; Frobell, 2011). The longitudinal design enabled evaluation of knee joint morphology and cartilage composition over time in the same group, which provides valuable information pertaining to the changes in joint morphology features and cartilage T2 values from 2.5-4.5 years following ACLR.

Second, ACLR participants were categorised into two groups; that is, those with and without concomitant meniscal pathology. This is particularly important given the increased risk of early-onset knee OA in patients with combined ACLR and meniscal pathology compared with isolated ACLR patients (Keays et al., 2010; Oiestad et al., 2009). Previous studies rarely compare joint morphology between individuals with isolated ACLR and those with concomitant meniscal pathology, and no study has investigated longitudinal changes in joint morphology amongst the two groups separately. To evaluate the effects of concomitant meniscal pathology in the
developing of premature knee OA, several morphological measures were included in the current study.

Third, to the author’s knowledge, the association between cartilage defects and subsequent cartilage volume change has not been examined in a post-traumatic ACLR cohort, and the association between BML size and cartilage volume change has only examined in the lateral compartment in ACLR patients. The initial injury to the articular cartilage and subchondral bone was believed to play an important role in post-traumatic OA (Nakamae et al., 2006); however, there is a lack of evidence to support that cartilage defects and BML lead to cartilage degenerative changes in ACLR patients. The current study for the first time found an association between cartilage defects score and subsequent cartilage volume increase in the ACLR cohort, supporting the notion that cartilage injury affects cartilage homeostasis leading to subsequent degeneration (Martin et al., 2006). The association between BML size and subsequent cartilage degeneration has been reported in the lateral compartment; by contrast, the current study also examined the association in the medial compartment (Frobell, 2011; Potter et al., 2012). The current study expanded the understanding of post-traumatic OA pathogenesis from the imaging perspective by examining these associations.

Fourth, this study is also the first study to investigate T2 cartilage values in ACLR participants at 2.5 years post-surgery and explored changes over the subsequent 2 years. Although previous longitudinal studies investigated the change of T2 value from 1 to 11 years following ACLR, quantitative data were only reported in the first two years (Li et al., 2011; Su et al., 2013). Furthermore, the inclusion of isolated ACLR patients increased the heterogeneity of the cohort and precluded the influence of combined meniscal pathology.
7.4 Limitations of the study

There are several limitations of the study. First, only 51% of the participants returned for follow-up assessment. This study utilised data from a longitudinal cohort study investigating the relationship between biomechanical factors and joint structure. Despite considerable efforts by the research team to minimise attrition, these young ACLR participants with busy lives failed to return for follow-up due to the time commitment required for biomechanical testing and MRI assessment (i.e., approximately 4 hours in total) as well as relocation. However, there was no difference in demographic features amongst those participants who remained in the study and those who dropped-out of the study.

Second, the sample size was relatively small for the combined ACLR and control groups. As mentioned above, the study was originally aimed to investigate the relationship between biomechanical factors and joint structure and was not specifically designed to address the research questions in this thesis, thus an a priori sample size calculation was not performed. Furthermore, there were no published studies reporting the cartilage volume in ACLR patients with and without meniscal pathology upon which to calculate sample size. Given the exploratory nature of the study, future studies with larger sample sizes and longer follow-up are required to confirm the results of this study.

Third, control participants were only assessed at baseline for the T2 mapping measurement in Chapter Six due to funding restrictions. As such, it is difficult to determine whether there are natural, time-related changes in T2 cartilage values among the healthy control participants over 2 years.
7.5 Future directions

Several directions for future research have emerged from the present findings. First, the current thesis assessed knee joint morphology and cartilage composition in ACLR patients at 2.5-4.5 years following surgery. It would be helpful to understand the disease process by investigating MRI-derived changes with longer follow-up. In addition, future studies of joint structural could be examined in the context of modifiable risk factors, including joint biomechanics and pro-inflammatory cytokine levels. Such information would be particularly useful to determine the risk of joint degeneration over different time periods.

Second, although the association between cartilage defects score and subsequent cartilage volume increase has been found in the ACLR patients with mild cartilage defects (grade 0-2), the influence of severe cartilage defects (grade 3-4) to joint structure is still unclear in post-traumatic OA. ACLR patients with severe cartilage defects have reported worse knee function compared with those with mild cartilage defects at 2-5 years post-surgery (Rotterud et al., 2012; Rotterud et al., 2013; Shelbourne et al., 2003); however, joint morphology and cartilage composition has not been thoroughly investigated. Theoretically, features of joint morphology and cartilage composition in ACLR patients with severe cartilage defects should be worse than those with mild cartilage defects. Furthermore, as mentioned above, various cartilage repair techniques have been used to manage cartilage defects, and future research is required to determine the efficacy of such treatment and develop more optimal therapies. MRI-derived joint morphology features and cartilage composition could be assessed non-invasively as the outcome in the clinical research.

Lastly, interventions aiming to slow structural joint degeneration should be further investigated in patients with ACL injury. Given the multifactorial nature of the OA disease, it is unlikely that a single management regimen will be effective. Optimal management of ACL injury has been proposed including rehabilitation, surgery,
education and biologic therapies, and the management of OA in physically active young adults requires load-reduction, activity modification, muscle strengthening and weight control (Bennell et al., 2012). Given that approximately 20% of ACLR patients sustain a subsequent ACL rupture in the same knee, efficacious rehabilitation programs focusing on the resolution of ACLR-related neuromuscular deficits could optimize the safe return to high-risk activity (Hewett et al., 2013). For ACLR patients with OA, concomitant high tibial osteotomy results in substantial changes in gait biomechanics at the time of reconstruction, and it could be beneficial for reducing the risk of OA (Marriott et al., 2015; Trojani et al., 2014). Biological therapies such as plate-rich plasma (PRP) has also been used in the treatment of ACL injury, and PRP could reduce maturation and integration time of graft following ACLR (Figueroa et al., 2015). Mesenchymal stem cell has been used for ACL regeneration in animal models and may also have benefits in ACL patients although this has not been widely investigated. Other potential therapeutic interventions including gene therapy, antibodies and inflammation inhibitors may gradually progress into clinical practice (Figueroa et al., 2014; Little et al., 2013). However, the clinical efficacy of these suggested treatments needs to be evaluated. Importantly, risk factors for OA progression are likely to be different according to the stage of the disease, for example, in the early OA stage ACLR knees may be underloading while in the late stage those knees are overloaded due to muscle weakness and impaired neuromuscular control. At this stage, there is no strong evidence to help guide clinicians as to effective treatments to slow structural degeneration in ACL-injured patients and should be a focus of future research efforts.
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Marriott, K., Birmingham, T. B., Kean, C. O., Hui, C., Jenkyn, T. R., & Giffin, J. R. (2015). Five-year changes in gait biomechanics after concomitant high tibial osteotomy and ACL reconstruction in patients with medial knee...


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## Appendix I

### Sports Activity Scale

**Check the box which describes your level of sports activity before your original knee injury.**

Then, check the box which describes your level of sports activity at this time.

<table>
<thead>
<tr>
<th>Level I (participates 4-7 days/week)</th>
<th>Level II (participates 1-3 days/week)</th>
<th>Level III (participates 1-3 times/month)</th>
<th>Level IV (no sports)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jumping, hard pivoting, cutting (basketball, volleyball, football, gymnastics, soccer)</td>
<td>Running, twisting, turning (tennis, racquetball, handball, ice hockey, field hockey, skiing, wrestling)</td>
<td>No running, twisting, jumping (cycling, swimming)</td>
<td>Jumping, hard pivoting, cutting (basketball, volleyball, football, gymnastics, soccer)</td>
</tr>
<tr>
<td>Running, twisting, turning (tennis, racquetball, handball, ice hockey, field hockey, skiing, wrestling)</td>
<td>No running, twisting, jumping (cycling, swimming)</td>
<td>Running, twisting, turning (tennis, racquetball, handball, ice hockey, field hockey, skiing, wrestling)</td>
<td>No running, twisting, jumping (cycling, swimming)</td>
</tr>
</tbody>
</table>

### Change in Sports Activities

**Check the box which best describes any change you have had in sports activities since your injury / surgery.**

My sports activities have:

- **Not Changed**
  - If yes, check one box below:
    - I have no / slight problems (c)
    - I have moderate / significant problems (d)
- **Decreased**
  - If yes, check one box below:
    - I now have no / slight problems (e)
    - I now have moderate / significant problems (f)
- **Stopped -- given up all sports**
  - If yes, check one box below:
    - I have moderate / significant problems when I play sports (f)
    - For reasons not related to my knee (g)

### Function ADL

**Check the problems you have during:**

1. **Walking**
   - check one box:
     - normal, unlimited
     - some limitations
     - only 3-4 blocks possible
     - less than 1 block; cane, crutch

2. **Stairs**
   - check one box:
     - normal, unlimited
     - some limitations
     - only 11-30 steps possible
     - only 1-10 steps possible

3. **Squatting / kneeling**
   - check one box:
     - normal, unlimited
     - some limitations
     - only 6-10 possible
     - only 0-5 possible

### Function Sports

**Check the problems you have during:**

1. **Straight running**
   - check one box:
     - fully competitive
     - some limitations, guarding
     - definite limitations, half speed
     - not able to do

2. **Jumping / landing on affected leg**
   - check one box:
     - fully competitive
     - some limitations, guarding
     - definite limitations, half speed
     - not able to do

3. **Hard twists / cuts / pivots**
   - check one box:
     - fully competitive
     - some limitations, guarding
     - definite limitations, half speed
     - not able to do

### Problems with Sports

**Describe the problems you would have with your knee after participating for one hour without guarding or limitations in each of the three sports categories below.**

- **Strenuous Sport** (soccer, football, basketball, volleyball)
  - check one box:
    - no problems
    - moderate problems during or after game
    - severe problems; cannot participate

- **Moderate Sport** (tennis, racquetball)
  - check one box:
    - no problems
    - moderate problems during or after game
    - severe problems; cannot participate

- **Light Sport** (golf, bowling, hiking)
  - check one box:
    - no problems
    - moderate problems during or after game
    - severe problems; cannot participate

---

**Total Points**

**Highest Level (before injury) ___ / 100**

**Highest Level (current) ___ / 100**

**Total Points __________**

**Highest Level (current) ____ / 100**

**Highest Level (before injury) ____ / 100**

---

**Sports Activity and Function Form**

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KOOS KNEE SURVEY

Today’s date: _____/_____/______ Date of birth: _____/_____/______

Name: ____________________________________________________

INSTRUCTIONS: This survey asks for your view about your knee. This information will help us keep track of how you feel about your knee and how well you are able to perform your usual activities. Answer every question by ticking the appropriate box, only one box for each question. If you are unsure about how to answer a question, please give the best answer you can.

Symptoms
These questions should be answered thinking of your knee symptoms during the last week.

S1. Do you have swelling in your knee?  
□ Never  □ Rarely  □ Sometimes  □ Often  □ Always

S2. Do you feel grinding, hear clicking or any other type of noise when your knee moves?  
□ Never  □ Rarely  □ Sometimes  □ Often  □ Always

S3. Does your knee catch or hang up when moving?  
□ Never  □ Rarely  □ Sometimes  □ Often  □ Always

S4. Can you straighten your knee fully?  
□ Always  □ Often  □ Sometimes  □ Rarely  □ Never

S5. Can you bend your knee fully?  
□ Always  □ Often  □ Sometimes  □ Rarely  □ Never

Stiffness
The following questions concern the amount of joint stiffness you have experienced during the last week in your knee. Stiffness is a sensation of restriction or slowness in the ease with which you move your joint.

S6. How severe is your knee joint stiffness after first wakening in the morning?  
□ None  □ Mild  □ Moderate  □ Severe  □ Extreme

S7. How severe is your knee stiffness after sitting, lying or resting later in the day?  
□ None  □ Mild  □ Moderate  □ Severe  □ Extreme
**Pain**

P1. How often do you experience knee pain?
- Never
- Monthly
- Weekly
- Daily
- Always

What amount of knee pain have you experienced the last week during the following activities?

P2. Twisting/pivoting on your knee
- None
- Mild
- Moderate
- Severe
- Extreme

P3. Straightening knee fully
- None
- Mild
- Moderate
- Severe
- Extreme

P4. Bending knee fully
- None
- Mild
- Moderate
- Severe
- Extreme

P5. Walking on flat surface
- None
- Mild
- Moderate
- Severe
- Extreme

P6. Going up or down stairs
- None
- Mild
- Moderate
- Severe
- Extreme

P7. At night while in bed
- None
- Mild
- Moderate
- Severe
- Extreme

P8. Sitting or lying
- None
- Mild
- Moderate
- Severe
- Extreme

P9. Standing upright
- None
- Mild
- Moderate
- Severe
- Extreme

**Function, daily living**

The following questions concern your physical function. By this we mean your ability to move around and to look after yourself. For each of the following activities please indicate the degree of difficulty you have experienced in the last week due to your knee.

A1. Descending stairs
- None
- Mild
- Moderate
- Severe
- Extreme

A2. Ascending stairs
- None
- Mild
- Moderate
- Severe
- Extreme
For each of the following activities please indicate the degree of difficulty you have experienced in the last week due to your knee.

A3. Rising from sitting
   None  Mild  Moderate  Severe  Extreme

A4. Standing
   None  Mild  Moderate  Severe  Extreme

A5. Bending to floor/pick up an object
   None  Mild  Moderate  Severe  Extreme

A6. Walking on flat surface
   None  Mild  Moderate  Severe  Extreme

A7. Getting in/out of car
   None  Mild  Moderate  Severe  Extreme

A8. Going shopping
   None  Mild  Moderate  Severe  Extreme

A9. Putting on socks/stockings
   None  Mild  Moderate  Severe  Extreme

A10. Rising from bed
    None  Mild  Moderate  Severe  Extreme

A11. Taking off socks/stockings
     None  Mild  Moderate  Severe  Extreme

A12. Lying in bed (turning over, maintaining knee position)
    None  Mild  Moderate  Severe  Extreme

A13. Getting in/out of bath
    None  Mild  Moderate  Severe  Extreme

A14. Sitting
    None  Mild  Moderate  Severe  Extreme

A15. Getting on/off toilet
    None  Mild  Moderate  Severe  Extreme
For each of the following activities please indicate the degree of difficulty you have experienced in the last week due to your knee.

A16. Heavy domestic duties (moving heavy boxes, scrubbing floors, etc)

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
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A17. Light domestic duties (cooking, dusting, etc)

<table>
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<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
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</table>

**Function, sports and recreational activities**
The following questions concern your physical function when being active on a higher level. The questions should be answered thinking of what degree of difficulty you have experienced during the last week due to your knee.

SP1. Squatting

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
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SP2. Running

<table>
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<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
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SP3. Jumping

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
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SP4. Twisting/pivoting on your injured knee

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
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SP5. Kneeling

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
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**Quality of Life**

Q1. How often are you aware of your knee problem?

<table>
<thead>
<tr>
<th>Never</th>
<th>Monthly</th>
<th>Weekly</th>
<th>Daily</th>
<th>Constantly</th>
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</table>

Q2. Have you modified your life style to avoid potentially damaging activities to your knee?

<table>
<thead>
<tr>
<th>Not at all</th>
<th>Mildly</th>
<th>Moderately</th>
<th>Severely</th>
<th>Totally</th>
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Q3. How much are you troubled with lack of confidence in your knee?

<table>
<thead>
<tr>
<th>Not at all</th>
<th>Mildly</th>
<th>Moderately</th>
<th>Severely</th>
<th>Extremely</th>
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</table>

Q4. In general, how much difficulty do you have with your knee?

<table>
<thead>
<tr>
<th>None</th>
<th>Mild</th>
<th>Moderate</th>
<th>Severe</th>
<th>Extreme</th>
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Thank you very much for completing all the questions in this questionnaire.
Appendix III

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