ERRATA

Amendments

Pg 45 Section 1.10 Aims of this thesis:

This thesis examines the effect of biomechanical and systemic risk factors on knee cartilage and bone and their change over time in both symptomatic/healthy subjects and in those with knee OA. More specifically this thesis examined:

1. The natural history of cartilage defects in subjects with knee OA
2. The prevalence and significance of meniscal tears and also the relationship between gait parameters and meniscal tears in a cohort of asymptomatic women.
3. The effect of biomechanical factors at the tibiofemoral and patellofemoral compartments in subjects with knee OA
4. The natural history and significance of bone marrow lesions in those with no clinical knee OA.
5. The relationship between systemic/vascular factors including smoking, dietary fatty acids and serum lipids and knee structure in asymptomatic participants.

Pg 167 Section 8.2.5.2 Determining Causation

Include the following after the 1st sentence:

In this study cartilage, bone and the menisci were examined. It is possible that other structural elements within the knee that can be visualised using MRI, including synovial and ligament changes may influence the development and/or progression of OA. However, they were not examined within this thesis and further work is required to assess their role.

Typographical Errors

Pg 5 'disuse of the TF compartment resulting...' to 'disuse of the TF compartment, resulting...'

Pg 5 'x-ray views to assess OA which restricted assessment' to 'x-ray views to assess OA, which restricted assessment'

Pg 5 'lateral x-ray views to epidemiological studies the involvement of the PF compartment...' to 'lateral x-ray views to epidemiological studies, the involvement of the PF compartment...'

Pg 5 'middle-aged adults with knee pain 40% of people had combined...to 'middle-aged adults with knee pain, 40% of people had combined'

Pg 5 'the knee is tri-compartmental joint' to 'the knee is a tri-compartmental joint'

Pg 17 'effected' to 'affected'

Pg 22 'non-gadadolinium' to 'non-gadolinium'

Pg 23 'a results of this' to 'a result of this'

Pg 25 'when they were follow up' to 'when they were followed up'

Pg 30 'Particularly as no association...' to '.., particularly as no association...'

Pg 30' predict loss of cartilage loss over time' to 'predict loss of cartilage over time'

Pg 31 'While in a predominantly OA-free population, progression of defects was associated with female gender...' to 'In a predominantly OA-free population, progression of defects was associated with female gender...'

Pg 32 'Whilst among participants...' to 'Among participants...'

Pg 32 'There is very limited data' to 'There are very limited data'

Pg 33, 34 and 35 'in addition' to 'in addition,'

Pg 35' in contrast' to 'in contrast,'

Pg 35 'Considering women have a greater risk of OA...' to 'Given women have a greater risk of OA...'

Pg 35 'As most research has been performed in populations who have undergone meniscectomy or have knee OA further research is required...' to 'As most research has been performed in populations who have undergone meniscectomy or have knee OA, further research...'

Pg 37 'It was also postulated' to 'These findings suggested'

Pg 38 delete 'among'

Pg 40 'then' to 'than'

Pg 49 'A six-camera Vicon motion analysis system (Oxford Metrics Ltd., Oxford, UK) was used to capture three-dimensional kinematic data during four walking trials on the dominant leg at the subjects' self-selected speed...' to 'A six-camera Vicon motion analysis system (Oxford Metrics Ltd., Oxford, UK) was used to capture three-dimensional kinematic data on the dominant leg during four walking trials, at the subjects' self-selected speed to capture normal gait patterns.'

Pg 51 'Each participant attended for a single morning fasting blood test at the time of the original study' to 'Each participant had a single morning fasting blood test at the time of the original study'

Pg 51 'Radiographs were obtained in for the population' to 'Radiographs were obtained for the population'

Pg 54 'Outcome measures in the study were knee structural variables measured using MRI, including knee cartilage volume and its rate of change, prevalence and grade of knee cartilage defects and its change in the respective compartment, tibial plateau bone area and its rate of change and incidence and persistence or resolution of BMLs.' To 'Outcome measures included knee cartilage volume and its rate of change, prevalence and grade of knee cartilage defects and its change in the respective compartment, tibial plateau bone area and its rate of change and incidence and persistence or resolution of BMLs.'

Pg 63 'There presence' to 'Their presence'

FACTORS AFFECTING THE KNEE JOINT IN HEALTH AND DISEASE: TOWARDS AN IMPROVED UNDERSTANDING OF KNEE OSTEOARTHRITIS

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A thesis submitted in fulfilment of requirements for the degree of

Doctor of Philosophy

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2010

Contents

Abstract

Osteoarthritis (OA) is a significant public health problem. It is the most common single cause of pain and disability in the elderly. OA is a complex disease that affects the whole joint. The knee is one of the joints most commonly affected by OA. The understanding of knee OA has been hampered by the lack of a sensitive tool to non-invasively assess disease severity. The ideal imaging modality for the assessment of OA would provide data pertaining to all joint structures, including a direct measure of both cartilage and bone, as well as other intra and extra-articular structures in three dimensions. Magnetic Resonance Imaging (MRI) promises to fulfil many of these criteria.

There is no known treatment for OA that stops the progression of the disease so current strategies are aimed only at relieving symptoms. There is evidence to suggest that the incidence and progression of knee OA may involve different mechanisms. The goal for researchers is to identify and understand the mechanisms of modifiable risk factors for OA in order to develop preventative strategies. The aim of this thesis was to address this by examining risk factors for structural changes in the knee that are associated with either the development or the progression of disease in both healthy/asymptomatic populations as well as in a population with knee OA. This provided the opportunity to examine these across the spectrum of disease from the normal joint through to one with OA.

Paper 1 presented within this thesis describes the natural history of cartilage defects and factors associated with the progression in those with knee OA. In this study, cartilage defects tended to progress over 2 years in people with symptomatic knee OA. Factors associated with progression of cartilage defects were increasing age and baseline tibial bone area.

Papers 2 and 3 describe the prevalence and significance of meniscal tears and also the relationship between gait parameters and meniscal tears in a cohort of asymptomatic post-menopausal women. Meniscal tears were found to be common and became more common with increasing age. Tears were also associated with

greater tibial plateau bone area, and prevalence of meniscal tears at baseline was weakly associated with decreased lateral cartilage volume and an increased progression of tibiofemoral cartilage defects over 2 years. In addition, gait parameters that isolate medial tibiofemoral joint loads were associated with medial meniscal pathology. The presence and severity of medial meniscal tears was positively associated with the peak external knee adduction moment during early stance, and tended toward a similar association during late stance. Moreover, the presence of medial meniscal lesions was also positively associated with the degree of internal foot rotation when the external knee adduction moment peaked during late stance, independent of the magnitude of the adduction moment.

Papers 4 and 5 explore local biomechanical factors affecting the tibiofemoral and patellofemoral compartments. In people with knee OA, a change in knee alignment from genu varum toward genu valgum over 2 years was associated with a reduction in the annual rate of medial tibial cartilage volume loss in the subsequent 2.5 years. Change in alignment did not affect the rate of change in lateral tibial cartilage volume. In addition within the patellofemoral compartment, a shallower femoral sulcus angle was associated with increased medial patella cartilage volume compared to a deeper femoral sulcus angle.

Papers 6 and 7 describe the natural history and significance of bone marrow lesions (BMLs) in healthy participants with no clinical knee OA. BMLs developed in 12% of people over 2 years. Increased weight and body mass index were risk factors for incident BMLs. Incident BMLs were also associated with the development of knee pain in a population where all participants were free of pain at the beginning of the study. Approximately half of the BMLs present at baseline resolved over the 2 year study period. In addition within this asymptomatic population, the development of new BMLs was associated with adverse effects on knee cartilage, while resolution of BMLs was associated with improvement in cartilage.

Papers 8, 9 and 10 examine the relationships between cigarette smoking, dietary fatty acids and serum lipids and BMLs in asymptomatic clinically healthy populations. In a cohort of asymptomatic, community based adults, a history of smoking (current and past) was associated with increased medial tibial, but not lateral tibial or patella

cartilage loss over 2 years. In addition there was a dose-response relationship between 'pack-years' smoked and increased medial tibial cartilage loss. For individuals who had a BML at baseline, smoking was associated with the persistence of the BML over 2 years. The persistence of the BML was found to partially mediate the relationship between smoking and cartilage loss. In the same population a higher intake of saturated fatty acids was found to be associated with an increased likelihood of developing BMLs over 2 years. In a cohort of asymptomatic middle-aged women with no clinical knee OA, serum cholesterol and triglyceride levels were found to be associated with the incidence of BMLs over 2 years.

This thesis examined the effect of biomechanical and systemic risk factors on knee cartilage, meniscal tears and bone and the significance of their change over time in both symptomatic/healthy subjects and those with knee OA. It identified a number of modifiable factors that influence changes indicative of disease development as well as disease progression. This thesis has contributed to the identification of knee structural changes in both the pre-diseased and diseased state as well as risk factors for these changes. Further work will be required to better understand the role of these different structural changes in the early disease and their associated risk factors in order to more effectively prevent and treat knee OA.

Publications Arising From Thesis

1. **Davies-Tuck ML**, Wluka AE, Wang Y, Teichtahl AJ, Jones G, Ding C, Cicuttini FM. The natural history of cartilage defects in people with knee osteoarthritis. **Osteoarthritis and Cartilage 2008; 16,** 337-42. (IF 3.554) (Ranked 3/48 in Orthopaedics discipline)

2. **Davies-Tuck M**, Teichtahl AJ, Wluka AE, Wang Y, Urquhart DM, Cui J, Cicuttini FM. Femoral sulcus angle and increased patella facet cartilage volume in an osteoarthritic population. **Osteoarthritis and Cartilage 2008; 16,** 131-5. (IF 3.554)

3. **Davies-Tuck ML**, Martel-Pelletier J, Wluka AE, Pelletier J-P, Ding C, Jones G, Davis SR and Cicuttini FM. Meniscal Tear and Increased Tibial Plateau Bone Area in Healthy Post-Menopausal Women. **Osteoarthritis and Cartilage 2008; 16,** 268-71. (IF 3.554)

4. **Davies-Tuck ML**, Wluka AE, Teichtahl AJ, Martel-Pelletier J, Pelletier J-P, Jones G, Ding C, Davis SR and Cicuttini FM. Association between Meniscal Tears and the Peak External Knee Adduction Moment and Foot Rotation During Level Walking in Postmenopausal Women without Knee Osteoarthritis: A Cross sectional Study. **Arthritis Research and Therapy 2008; 10,** R58. (IF 4.49)

5. **Davies-Tuck ML** & Teichtahl, Andrew; Wluka AE, Jones G, and Cicuttini FM. Change in knee angle influences the rate of medial tibial cartilage volume loss in knee osteoarthritis. **Osteoarthritis and Cartilage 2009; 17,** 8-11. (IF 3.554)

6. **Davies-Tuck ML**, Wluka AE, Wang Y, English DR, Giles GG, and Cicuttini FM. The Natural History of Bone Marrow Lesions in Community Based

Adults with no clinical knee osteoarthritis. **Annals of the Rheumatic Diseases 2009; 68**(6):904-8 (IF 7.188- 1st in Rheumatology)

7. **Davies-Tuck ML**, Wluka AE, Forbes A, Wang Y, English DR, Giles GG & Cicuttini F. Smoking is Associated with Increased Cartilage Loss and Persistence of Bone Marrow Lesions over 2 Years in Community Based Individuals with No Clinical Knee Osteoarthritis. **Rheumatology 2009 48**(10): 1227-31 (IF 4.052)

8. **Davies-Tuck ML** & Wang Y, Wluka AE, English DR, Giles GG, O'Sullivan R and Cicuttini F. Dietary Fatty Acid Intake affects the risk of developing Bone Marrow Lesions in Healthy Middle-Aged Adults without Knee Osteoarthritis. **Arthritis Research and Therapy 2009 11**(3)**:** R63 (IF 4.49)

9. **Davies-Tuck ML**, Wluka A, Forbes A, Wang Y, English DR, Giles GG & Cicuttini F Development of Bone Marrow Lesions is associated with adverse effects on knee cartilage while resolution is associated with improvement-a potential target for prevention of knee osteoarthritis: a longitudinal study. **Arthritis Research and Therapy 12: R10**

10. **Davies-Tuck ML,** Fahad Hanna, Davis SR, Wluka AE, Bell R, Adams J & Cicuttini FM. Increased Total Cholesterol and Triglycerides are Associated with the Incidence of Bone Marrow Lesions in Asymptomatic Middle-Aged Women **Arthritis Research and Therapy 2009 11**(6): R181

Publications not directly related to thesis

1. Wluka AE, Wang Y, **Davies-Tuck ML**, English DR, Giles GG and Cicuttini FM. BML predict increase in cartilage defects and loss of cartilage volume in healthy middle-aged adults over 2 years. **Rheumatology 2008; 47,** 1392-6. (IF 4.045)

2. Berry P, **Davies-Tuck ML**, Wluka AE, Hanna F, Bell R, Davis SR, Adams J and Cicuttini FM. The Natural History of BMLs in Community-

based Middle-Aged Women with no Clinical Knee Osteoarthritis. **Seminars in Arthritis and Rheumatism, 2009 39**(3): 213-7 (IF 3.44)

3. Wluka AE, Hanna F, **Davies-Tuck ML**, Wang Y, Bell R, Davis SR, Adams J and Cicuttini FM. Bone Marrow Lesions predict increase in knee cartilage defects and loss of cartilage volume in healthy middle-aged women over 2 years **Annals of the Rheumatic Diseases 2008; 68**(6): 850-5. (IF 7.188-1st in Rheumatology).

4. Teichtahl, A.J, Wluka, A.E, **Davies-Tuck, ML** and Cicuttini, F.M. Imaging Of Knee Osteoarthritis, **Best Practice Clinical Rheumatology 2008; 22,** 1061-1074. (IF 2.088)

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I wish to express my sincere thanks to Associate Professor Andrew Forbes for not only his statistical help but also for the support and encouragement he has offered me. Many thanks also go to Ms. Judy Hankin, Dr Yuanyuan Wang, Dr Fahad Hanna, Dr. Donna Urquhart, Dr. Andrew Teichtahl, Professor Susan Davis, Associate Professor Robin Bell, Dr. Changhai Ding, Professor Graeme Jones, Ms Sharon Brennan and Ms Stephanie Tanamas for their valuable advice and contributions. Thankyou to all those who have kindly helped me during the preparation and completion of this thesis.

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I would like to especially thank the participants of the studies, for without them none of this would have been possible.

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Finally I reserve my deepest gratitude to my family; to my parents for instilling the value of education and for all their encouragement and support over the years, to my husband Paul, for his love, understanding and support and to my son Luke, who arrived at the end of this journey.

Thank you

Declaration

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma, except where due reference is made in the text of the thesis.

To the best of my knowledge, this thesis contains no material previously published or written by another person except where due reference is made in the text of the thesis.

PART A: General Declaration

Monash University
Monash Research Graduate School

Declaration for thesis based or partially based on conjointly published or unpublished work

General Declaration

In accordance with Monash University Doctorate Regulation 17/ Doctor of Philosophy and Master of Philosophy (MPhil) regulations the following declarations are made:

I hereby declare that this thesis contains no material which has been accepted for the award of any other degree or diploma at any university or equivalent institution and that, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

This thesis includes 9 original papers published in peer reviewed journals and 1 unpublished publications. The core theme of the thesis is 'Factors affecting the knee joint in health and disease'. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the Department of Epidemiology and Preventive Medicine under the supervision of Professor Flavia Cicuttini and Dr. Anita Wluka.

The inclusion of co-authors reflects the fact that the work came from active collaboration between researchers and acknowledges input into team-based research.

In the case of Chapters 3-7 my contribution to the work involved the following:

Thesis chapter	Publication title	Publication status*	Nature and extent of candidate's contribution
3	The natural history of cartilage defects in people with knee osteoarthritis	Published	Literature review, data analysis and interpretation, manuscript preparation
4	Meniscal Tear and Increased Tibial Plateau Bone Area in Healthy Post-Menopausal Women	Published	Literature review, data analysis and interpretation, manuscript preparation
4	Association between Meniscal Tears and the Peak External Knee Adduction Moment and Foot Rotation During Level Walking in Postmenopausal Women without Knee Osteoarthritis: A Cross sectional Study	Published	Literature review, data analysis and interpretation, manuscript preparation
5	Change in knee angle influences the rate of medial tibial cartilage volume loss in knee osteoarthritis	Published	Literature review, data analysis and interpretation, manuscript preparation
5	Femoral sulcus angle and increased patella facet cartilage volume in an osteoarthritic population	Published	Literature review, measurement of sulcus angle, data analysis and interpretation, manuscript preparation
6	The Natural History of Bone Marrow Lesions in Community Based Adults with no clinical knee osteoarthritis	Published	Literature review, measurement of bone marrow lesions, data analysis and interpretation, manuscript preparation

6	Bone Marrow Lesions are associated with increased cartilage loss, progression of defects and bone expansion over 2 years in Community Based Individuals with No Clinical Knee Osteoarthritis	Published	Literature review, measurement of bone marrow lesions, data analysis and interpretation, manuscript preparation
7	Smoking is Associated with Increased Cartilage Loss and Persistence of Bone Marrow Lesions over 2 Years in Community Based Individuals with No Clinical Knee Osteoarthritis	Published	Literature review, measurement of bone marrow lesions, data analysis and interpretation, manuscript preparation
7	Dietary Fatty Acid Intake affects the risk of developing Bone Marrow Lesions in Healthy Middle-Aged Adults without Knee Osteoarthritis	Published	Literature review, measurement of bone marrow lesions, data analysis and interpretation, manuscript preparation
7	Increased Total Cholesterol and Triglycerides are Associated with the Incidence of Bone Marrow Lesions in Asymptomatic Middle-Aged Women.	Published	Literature review, measurement of bone marrow lesions, data analysis and interpretation, manuscript preparation

[* For example, 'published'/ 'in press'/ 'accepted'/ 'returned for revision']

I have renumbered sections of submitted or published papers in order to generate a consistent presentation within the thesis.

Signed:

Date:

Abbreviations

ACL	Anterior cruciate ligament
ACR	American College of Rheumatology
AP	Antero-Posterior
BLOKS	Boston Leeds Osteoarthritis Knee Score
BOKS	Boston Knee Osteoarthritis Study
BMD	Bone mineral density
BMI	Body mass index
BMLs	Bone marrow lesions
CI	Confidence interval
COX-2	Cyclooxygenase-2
DALYs	Disability Adjusted Life Years
ERT	Estrogen Replacement Therapy
JSN	Joint Space Narrowing
JSW	Joint Space Width
KL	Kellgren-Lawrence
KOSS	Knee Osteoarthritis Scoring System
MOST	Multi-centre Osteoarthritis Study
MRI	Magnetic Resonance Imaging
NHANES	National Health and Nutrition Examination Survey
OA	Osteoarthritis
NSAIDs	Non-steriodal Anti-inflammatory Drugs
PF	Patello-femoral
ROA	Radiographic osteoarthritis
SEM	Southeast Michigan study
TF	Tibio-femoral
WOMAC	Western Ontario and McMaster Osteoarthritis Index
WORMS	Whole Organ Magnetic Resonance Imaging Score

Chapter 1: Introduction

1.1 Organisation of Thesis

Chapter 1 gives an overview of this thesis including its organisation, followed by a literature review, and the aims of this thesis.

Chapter 2 provides a more detailed description of the methodology used. It gives the details of the study populations, data collected, outcome measures and statistical analyses.

Chapters 3-7 present the results of this thesis.

Chapter 8 presents an overall discussion that integrates the findings in this thesis.

Chapter 9 provides a conclusion that summarises the key findings of this thesis

1.2 What is Osteoarthritis?

1.2.1 Definition

Osteoarthritis (OA) is a complex disease that affects the whole joint. While it is characterised by loss of articular cartilage and subchondral bone, periarticular tissues such as synovium, tendons, ligaments and muscles are also implicated. OA involves not only axial, but also peripheral joints; those that bear weight (such as the hips and knees, and the first metatarsophalangeal joint) as well as those do not (such as the interphalangeal joints, and the shoulders). The most widely used definition of OA is from the American College of Rheumatology (ACR), which defines OA as "a heterogenous group of conditions that lead to joint symptoms and signs, which are associated with defective integrity of articular cartilage in addition to related changes in the underlying bone margins" [1].

1.2.2 Prevalence

OA is the most common joint disease. It affects over 1.3 million Australians or 6.7% of the population with an estimated 40,000 new cases occurring per year [2]. This figure is expected to increase dramatically. It has been estimated that by the year 2050 the prevalence of OA will reach 10.7% or 3.14 million people [3]. The significant impact of arthritis and other musculoskeletal diseases was recognised in July 2002, when musculoskeletal diseases were declared to be the 7th National Health Priority Area.

1.2.3 Impact

OA is a significant public health problem in developed countries as it is the most common single cause of disability [4]. It has a severe impact at both the individual and population level.

The financial impact is staggering. In 2004–05, Australia spent just under $4 billion on arthritis and other musculoskeletal conditions, of which OA accounted for the largest proportion of direct health expenditure at $1.2 billion (31%) [2]. The direct

health expenditure on OA was more than 2.3% of the total allocated health expenditure in Australia [5]. In 2007-8 OA was one of the top 10 problems managed by GPs with almost 2.7 million Medicare-paid GP consultations recorded [2]. Unfortunately there is no known treatment that stops the progression of the disease [4, 6-8] and joint replacement is the final treatment for end stage disease. The cost of knee and hip replacements in 2004-05 in Australian public hospitals alone, was $145 million and $186 million respectively, representing over 20% of the total inpatient cost for arthritis in Australia [3].

The financial costs are only one aspect. OA is also the leading cause of pain and disability among the elderly, it represents the 3rd largest contributor to years of life lost due to disability in Australia [9]. In 2004 it was estimated that patients with OA had incurred 67,460 DALYs (disability adjusted life years) lost [10], representing 79% of the total arthritis burden of disease. While mortality rates associated with OA are relatively low, patients with OA experience a substantially lower quality of life when compared to the people without OA [10]. In addition compared to any other disease, OA accounts for more dependency in walking, stair climbing and other lower extremity tasks [11].

1.3 Clinical Features of Osteoarthritis

1.3.1 Symptoms

The main symptom of OA is pain that is aggravated by activity [12]. Pain is the predominant reason patients with OA initially see their doctor [13]. OA of the weight-bearing joints (i.e. hip and knee) can also lead to altered gait which often occurs due to the patients' attempt to protect the afflicted joint [6, 14]. Instability and buckling is another sign of OA, for example patients with knee OA may present with ligament laxity or deficiency [6, 14]. Most of this is not due to problems with the integrity of ligaments but rather altered joint morphology and muscle changes. Swelling of the joint may also occur due to synovitis, synovial effusion or abnormal bony enlargement which can lead to a loss of extension/function [6]. Grinding, crunching or crackling, also known as crepitus, may be heard over a joint with OA. This noise is

reflective of the uneven surfaces rolling across each other and may be present in both active and passive motion of the joint [6]. Other symptoms that can be reported include stiffness, tenderness of periarticular soft tissue and a warm sensation in the joint. Patients also report a loss of function and/or difficulty in ambulation [6].

1.3.2 Diagnosis

The diagnosis of OA involves a combination of clinical features and radio-graphical appearance. The ACR has produced criteria for diagnosis of OA [15]. The criteria encompasses a number of features including pain, morning stiffness, crepitus, bony enlargement and increased age as well as radiological evidence [6]. Radiological evidence of disease includes joint space narrowing (JSN), where the distance between two articular surfaces has decreased as a consequence of disease [16]. This can progress until the joint space is totally lost. Other features include osteophytes (which are bony spurs that occur on the surface of subchondral bone [17]), as well as subchondral cysts and subchondral sclerosis. The current methods for diagnosing OA are not without limitations and there is evidence of a discordance between clinical features and radiographic evidence of OA[18, 19].

1.3.3 Treatment

There is no known treatment for OA that stops the progression of the disease, current strategies are aimed at relieving symptoms [4, 6-8]

Treatments for OA include; non-pharmacological methods such as patient education, weight management, an increase of physical activity, use of mechanical aids and supplements such as glucosamine sulfate and chondroitin sulfate [7, 20]. These approaches are usually described in the management of early stage disease. As the disease and symptoms progress, drug therapy is frequently administered. Pharmacological treatments for OA include oral analgesics such as paracetamol, opioids, non-steroidal anti-inflammatory drugs (NSAIDs) and COX-2 (cyclooxygenase-2) inhibitors [7, 20]. Intra-articular injections and topical treatments to ease pain may also be administered [20]. The final treatment for severe end-stage disease is referral to an orthopaedic surgeon. Surgical interventions include;

arthroscopic debridement, osteotomy or total joint replacement. While joint replacement is a radical treatment, the surgery can result in excellent outcomes in terms of both improving the patient's quality of life and restoring joint alignment [7, 20, 21].

1.4 Knee Osteoarthritis

The knee joint is most commonly affected by OA and the knee OA is the most frequent cause of chronic disability in the elderly [10, 11, 22]. The knee is a tri-compartmental joint. It is comprised of the medial and lateral tibiofemoral (TF) and the patello-femoral (PF) compartment. The TF compartment is formed by the articulation of the femoral and tibial condyles and allows full extension to almost complete flexion, internal and external rotation as well as adduction and abduction [23]. The PF compartment is formed by the articulation of the under surface of the patella and the femur head. The patella has a dual role as both an articulation point and a component of the knee extension apparatus [24]. The patella lengthens the lever arm of the quadriceps muscle throughout the knees range of motion, allows a wider distribution of compressive stress on the femur, acts as a guide for the quadriceps tendon in centralising the input from the four aspects of the quadriceps muscles, controls capsular tension of the knee as well as acting as a bony shield to protect trochlear and condylar cartilage [25, 26]. It is the differing functions of the compartments within the knee that make it unique.

Traditionally OA was thought to be a disease of the TF compartment, resulting in the PF compartment being largely ignored. This was most likely due to the use of anteroposterior (AP) x-ray views to assess OA, which restricted assessment to the TF compartment [27, 28]. However, with the incorporation of skyline or lateral x-ray views to epidemiological studies, the involvement of the PF compartment has been revealed [29, 30]. PF OA can exist in the absence of TF disease [24], however they are commonly seen together [31, 32]. In a large population based study of middle-aged adults with knee pain, 40% of people had combined TF/PF OA, 24% had isolated PF OA and 4% had isolated TF OA [31]. Among patients who had undergone meniscectomy, combined TF and PF OA was present in 18% of knees, 3% had

isolated PF OA and 98% had isolated TF OA [32]. The designs of the TF and PF compartments each reflect unique functions and mechanics [25] and the same activity will have different effects on the different compartments. Consequently there are different radiographic patterns of knee OA [33]. While TF disease is more common in the medial compartment PF disease is more common in the lateral compartment [34-38].

While the prevalence of knee pain and symptoms rises with increasing radiographic severity of disease [18, 19, 39-42], the source of pain in knee OA is unclear. Pain has been found to predominantly emanate from the PF compartment. Among symptomatic women, PF OA was associated with higher levels of disability than TF OA [24]. In a population with predominantly mild OA, osteophytes within the PF compartment were associated with pain, while those in the TF compartment were not [43]. In a cross-sectional study of 133 women with knee pain, loss of patella cartilage assessed by MRI was also associated with increased pain, function and global Western Ontario and McMaster osteoarthritis index (WOMAC) scores, while loss of tibial or femoral cartilage was not [44].

Although their functions and pattern of OA may be different they also share some common risk factors.

1.5 Risk Factors for Knee Osteoarthritis

Many factors have been shown to be associated with an increased prevalence and/or incidence of knee OA. They can be broadly described as either modifiable or non-modifiable and they may act via biomechanical or systemic mechanisms. There is evidence to suggest that the incidence and progression of knee OA may involve different processes. For example, while the prevalence of knee OA is high within the community, not all of these people go on to need a joint replacement. Furthermore studies of disease progression have shown that disease may remain stable for many years in some patients, while it progresses very quickly in others [27, 45-48]. The preventive strategies for OA should focus on the modifiable risk factors for both the incidence and progression of disease.

1.5.1 Non-modifiable Risk Factors

Non–modifiable risk factors for OA include age, gender, ethnicity and genetics and are discussed below.

1.5.1.1 Age

Increasing age has consistently been related to an increased prevalence of both radiographic and symptomatic knee OA [49-54]. The occurrence of symptomatic knee OA under the age of is 50 un-common, however the rates significantly increase over the age of 50 [50]. Among the elderly participants within the Framingham population, the prevalence and severity of radiographic OA (ROA) increased with age [53].

While the aetiology of this age related increase is unclear, it has been suggested that the aging cartilage may be more susceptible to fatigue fractures [55] or it is possible that increased subchondral stiffness or neuromuscular changes explain this relationship [55, 56].

1.5.1.2 Gender

Knee OA has been shown to be more prevalent in females than in males [57]. In a radiographic survey of people in Leigh aged 55 to 64 years, 87% of females and 83% of males had radiographic OA [57]. The female gender has also been associated with an increased incidence of OA [53] as well as increased progression of disease [58]. Women have been shown to have a 1.5-4 times increased risk of knee OA compared to men [59]. Within the Framingham population, women had an increased risk of developing knee OA [58] and in a population based study of the progression of ROA, women had more severe progression of cartilage loss than men over 12 years [53]. Women also have twice the risk of bilateral knee OA that men have [50]. The mechanism by which the female gender increases the risk of knee OA is unknown however it may be a combination of hormonal and biomechanical differences between men and women.

1.5.1.3 Genetics

Genetic factors have been found to be strong determinants of knee OA. Familial clustering of hand and knee OA has been observed [52]. The findings from a large twin study of 130 identical and 120 non-identical twins suggested that the heritability component of knee OA may be around 40-50% [60]. Heritability has also been found to be stronger in females [61, 62]. While a single gene defect has not been identified for generalised OA [63] findings from animal, rare cases of familial OA and linkage studies have suggested some potential culprits. These include genes that code for cartilage and bone constituents including proteins involved in cartilage and bone turnover as well as inflammatory processes (discussed in[63]) however as yet results are inconclusive.

1.5.1.4 Ethnicity

While all races can be affected by OA, ethnic differences in knee OA have been observed. Higher rates of knee OA were observed in African-American women when compared to Caucasians in The National Health and Nutrition Examination Survey (NHANES) [64]. African-Americans with knee OA also had more severe radiographic features of disease and more frequent bilateral involvement and mobility impairment than Caucasians [65]. The Beijing Osteoarthritis Study reported an increased prevalence of knee OA among Chinese women when compared to Caucasian women in the Framingham study [66].

The mechanisms by which ethnicity affects a person's risk of OA is not known and the relative contributions of biological, lifestyle, genetic and socioeconomic factors to ethnic differences in OA are unclear.

1.5.2 Modifiable risk factors

In order to develop preventative strategies, the goal for researchers is to identify and understand the mechanisms of modifiable risk factors for OA. To date a number of modifiable risk factors have been identified. These include: obesity, joint alignment, trauma, meniscal pathology, physical activity, occupation, muscle weakness,

hormonal status, bone density and nutritional factors. These are discussed further below.

1.5.2.1 Obesity

Obesity has been recognised as the strongest modifiable risk factor for knee OA. It has been shown to increase both the prevalence and incidence of disease [49, 57, 64, 67-69]. Cross-sectional studies have consistently shown an association between obesity and knee OA, which has generally been stronger in women than in men. The reported increased risks range from 2- to 7-fold for women in the top tertile of body mass index (BMI) compared to women in the bottom tertile [49, 57, 64, 67].

Longitudinal studies have also consistently shown an association between obesity and knee OA. In the Framingham study, a strong association between being overweight in 1948-1951 and having knee OA approximately 36 years later was found. This association was stronger for women than men. Furthermore, this association was present for both symptomatic and asymptomatic disease [68]. The Johns Hopkins Precursors Study demonstrated a dose response relationship for increased BMI at a young age with an increased risk of knee OA in men[69]. The Chingford Study in middle-aged women found over a four year period that the odds of developing incident osteophytes, but not JSN was significantly increased in those in the highest tertile for BMI [70]. These results suggest that a higher BMI earlier in life may be a more important risk factor for the subsequent development of knee OA than heavier weight later in life. In persons who are overweight, weight loss can reduce the risk for OA [71].

The mechanism by which obesity increases the risk of knee OA is unclear, but both systemic and local biomechanical factors have been implicated.

1.5.2.2 Joint Alignment

Mal-alignment of the lower leg, in either the valgus or varus direction, has been found to influence the distribution of load across the articular surfaces of the knee joint [72]. Minor alterations in knee alignment have been shown to result in abnormal load

distribution across the joint [73-75]. The varus-load-bearing axis passes through the medial compartment, while the valgus passes through the lateral compartment [72]. It is thought that these increases in compartment loading increase stress on articular cartilage and other joint structures, subsequently leading to degenerative changes.

Recent studies examining the relationship between malalignment and knee OA have produced conflicting results [75]. Among 121 adults with symptomatic knee OA, increasing varus knee alignment was associated with an increasing risk of medial compartment joint space narrowing and osteophytes, while increasing valgus knee alignment was associated with an increased risk for lateral compartment joint space narrowing and osteophytes [73]. Similarly in patients with knee OA, varus alignment was shown to correlate with disease severity [74]. However, findings from a nested-case control study within the Framingham cohort revealed that baseline knee alignment was not associated with the incidence of knee OA [76].

The relationship between alignment and disease progression is clearer [76]. In a large longitudinal study of primary knee OA, varus knee alignment was associated with a 4-fold increase in medial progression, while valgus alignment was associated with a 5-fold increase in lateral progression over 18 months [76]. Similar findings were observed among 693 varus knees examined in the Rotterdam study, where varus knee alignment was also associated with medial OA progression over 6.6years [77].

Varus-valgus alignment also influences joint forces within the PF compartment [35, 36]. Among subjects with PF OA, in those with lateral PF OA 57% had valgus malalignment while only 24% of patients with medial PF OA did. Valgus alignment was also associated with lateral PF narrowing while varus alignment was associated with medial PF narrowing [36]. Consistent with this in patients with knee OA, varus alignment increased the odds of medial PF progression and while valgus alignment increased the progression of lateral PF OA[78].

Whether altered alignment predicts disease or is just a marker of disease is yet to be revealed.

1.5.2.3 Trauma

Non-specific knee injury has been demonstrated to significantly increase the risk of knee OA [79, 80]. It has been estimated that this is the most significant modifiable risk factor for men, and is second to obesity in women [18]. Anterior cruciate ligament (ACL) rupture has been associated with subsequent knee OA; 50-75% of patients develop knee OA 15-20 years following ACL rupture combined with associated injuries[81, 82]. It has been hypothesized that the mechanism by which ACL rupture facilitates the development of subsequent knee OA is by knee instability, which causes meniscal damage; which has been suggested to play a synergistic role in increasing the risk of knee OA.

1.5.2.4 Meniscal Pathology

Meniscal pathology significantly increases the risk of subsequent knee OA [83]. This was first described by examining cohorts of subjects following total meniscectomy [84]. Subsequent studies have confirmed the trend of these results, despite changes in surgical method. However, the magnitude of the increased risk varies between studies, probably due to differences in the: definition of OA, aetiology of meniscal tear, extent of meniscal injury, variation in associated injury, duration of follow-up, small sample size and choice of controls [21]. In a cohort study of post-meniscectomy subjects with a 16 year follow-up, the relative risk of radiographic OA following meniscectomy was increased at 4.8 (95% confidence interval (CI) 2.2-12.0) [83]. The main role of the meniscus is shock absorbing and if damaged or removed, results in increased contact stress within the knee joint. It is thought that this increase in contact stress to articular cartilage and subchondral bone may initiate the development of OA with early degenerative meniscal damage being a signal of early osteoarthritic disease [85].

1.5.2.5 Physical Activity

The effect of physical activity on the risk of knee OA is controversial. A small longitudinal study of marathon runners showed no effect of running on OA [86, 87]. Another cohort study of long distance runners aged over 50 years suggested that runners had more sclerosis and osteophyte formation than community controls [88,

89]. However, there were no differences in symptoms or JSN. Surveys attempting to relate self-reported knee OA and habitual physical activity have found no effect [90, 91]. In contrast, data from the Framingham study suggested increased recreational physical activity was associated with increased risk of knee OA [58, 92]. A prospective study in Bristol suggested regular participation in sport increased the risk of knee OA[93].

Strenuous, high-intensity, repetitive activity has more consistently been associated with the development of knee OA [94-96]. The effect is not sport specific; a study of Finnish former elite athletes showed an increase in hospital admission diagnoses of OA for athletes from all types of sports compared to an age matched population [96]. A study examining the risk of disease in previously elite tennis players and runners, which compared them with community controls showed an increase in knee OA in former elite athletes only [96].

Physical activity does not appear to affect the risk of progression of knee OA. Runners aged over 50 were followed for 5 years to identify whether running affected progression of OA: no effect was seen [89]. No effect was also noted on progression of disease in the Bristol cohort [93].

1.5.2.6 Occupation

Physically active occupations which involve knee bending, squatting or kneeling, increase the risk for knee OA [57, 64, 97-99]. Miners and dockers have a higher prevalence of knee OA than those in sedentary jobs [57, 97]. The first NHANES showed an association between knee bending demands and knee OA in persons aged 55 to 64 years [64]. Analysis of data from the Framingham study, showed a relationship between occupational physical labor, knee bending and later OA, especially among men [98]. A case-control study in the UK showed an increased risk of knee OA in those whose main job entailed more than 30 minutes per day of squatting or kneeling or climbing more than ten flights of stairs per day. The increase in risk associated with kneeling or squatting was more marked in subjects whose jobs entailed heavy lifting [99].

1.5.2.7 Muscle Weakness

Quadriceps weakness has long been recognized as a common feature in knee OA [100, 101]. The Beijing OA Study, a cross-sectional study involved 2,472 subjects ages 60 years or over, suggested a relationship between quadriceps weakness and knee OA in all compartments [102]. It has been unknown until recently whether this weakness occurred as a result of OA or preceded it. A prospective study following elderly subjects, showed that those with muscle weakness were more likely to develop knee OA than those without [100]. However, when the same study subjects were examined longitudinally, there was no significant difference in quadriceps strength between subjects with radiographically stable OA and those for whom joint damage progressed, suggesting that factors other than quadriceps weakness are more important determinants of OA progression [103]. It is likely that increased quadriceps strength may protect against OA onset, but may not delay the progression of the disease, as cartilage becomes increasingly abnormal.

1.5.2.8 Hormonal Status and Bone Density

The high incidence of OA in women after menopause suggests the role of estrogen deficiency in causing OA. Cohort studies have shown that estrogen replacement therapy (ERT) decreased the prevalence [104] and incidence [105, 106] of radiographic knee OA, suggesting a protective effect of ERT. However, the evidence remains conflicting. Some studies found that ERT was associated with a higher prevalence of knee OA [107-109], while others found that ERT was not significantly associated with the prevalence of OA [110].

Cross-sectional studies have demonstrated that women with knee OA appear to have high bone mineral density (BMD) [111], and that increasing knee OA scores (Kellgren and Lawrence grading scale) were associated with increasing BMD [112]. A report from the Framingham study suggested that high BMD decreased the risk of progression of radiographic knee OA, but increased the risk of incident knee OA [30].

1.5.2.9 Nutritional Factors

Results from the Framingham study suggested that higher dietary vitamin C, vitamin E and beta-carotene intake reduced the risk of progression of radiographic knee OA, but not the incidence knee OA [113, 114]. Low intake and low serum levels of vitamin D were shown to be associated with an increased risk for progression of radiographic knee OA. However, intake or serum levels of vitamin D were not associated with incident OA of the knee [114].

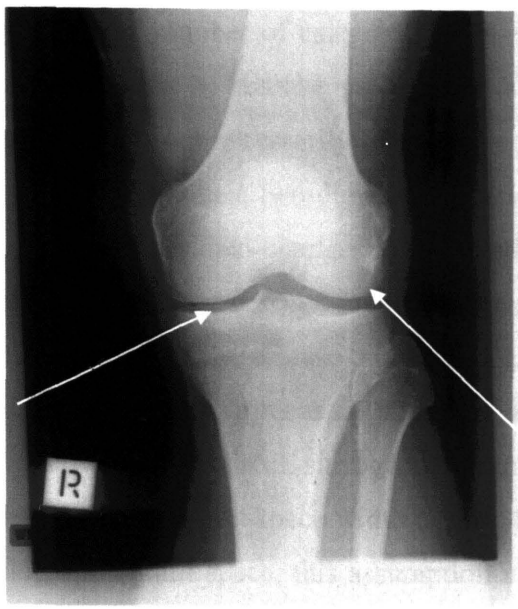
1.6 Imaging the Knee Joint and Measuring Disease

The understanding of knee OA has been hampered by the lack of a sensitive tool to non-invasively assess disease severity. This has meant that disease is only identified when significant pathology is present and visible on a radiograph. While radiographs are considered the current gold standard [115], they are not without limitations. Magnetic resonance imaging (MRI) is a more modern imaging modality which allows a three-dimensional assessment of the entire joint, and has offered a quantitative alternative to radiographs for examining the pathogenesis of OA [116-118]. However, the utility of MRI in the assessment of knee OA has been restricted to research settings only. Figure 1 demonstrates the different features that can be seen by x-ray compared to MRI.

1.6.1 Radiographic Assessment of Knee Osteoarthritis

X-rays remains the simplest and least expensive method to image OA of the knee [119] and is the current gold standard for assessing OA in both clinical and epidemiological settings. Radiographic assessment of knee OA involves scoring the severity of structural change in the knee from antero-posterior (AP) knee radiographs. In 1957 Kellgren and Lawrence developed the first standardized scoring system of radiographic knee OA [57], their system was then later adopted by others as the standard measure for assessing radiographic OA [1]. Their method involves grading the individual features of OA including joint space narrowing JSN (as a surrogate for articular cartilage reduction), osteophytes, subchondral bone changes, and bony

A



B – Sagittal image, T1 weighted

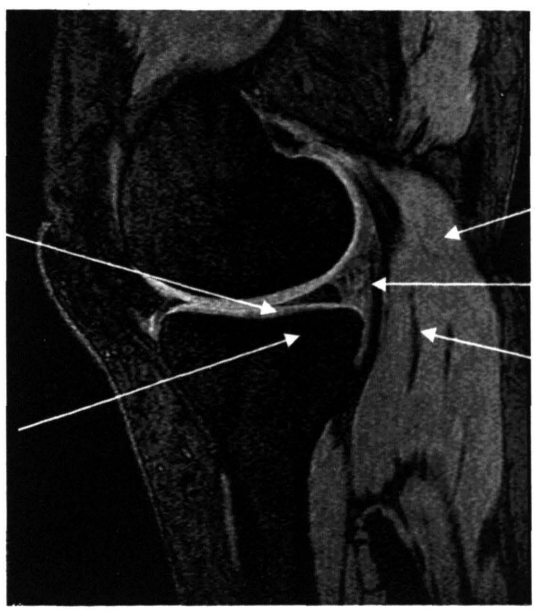


Figure 1. A Comparison of the information that can be obtained from a knee X-Ray (A) and MRI (B)

attrition [1]. However, there are several limitations associated with the use of these grading systems. Firstly, these grading systems predominantly employ ordinal measures, with only a limited number of categories. These therefore yield relatively crude and insensitive measures of disease progression and small changes in the individual features assessed do not necessarily translate into a change in grade [119]. Radiographs are also limited by their two-dimensional assessment of bony features and cannot visualise three-dimensional changes in intra and extra-articular structures, such as subchondral bone, cartilage defects, menisci, ligaments and synovial tissues that are now recognised as part of the disease process [119, 120]. Furthermore, while expert consensus has suggested that, a measure of cartilage should be used [115] the joint radiograph only provides an indirect, surrogate measure of articular cartilage via an assessment of the radiological joint space width (JSW). Since other structures also exist within the radiographic joint space, this assumption may not always be correct. Indeed, meniscal extrusion has been shown to account for much early joint space loss [121].

In addition there are further limitations associated with the grading of the individual features assessed by radiograph. These are discussed below.

1.6.1.1 Joint Space Narrowing

JSN is a continuous measure that has been employed as the outcome in studies of disease progression of knee OA [122]. The measurement can be made manually, with the use of callipers or a simple graded ruler and a micrometric eye piece, or semi automated, with the use of computer software [123]. The underlying assumption of using JSN is that a longitudinal reduction in joint space is a valid surrogate measure of a reduction in articular cartilage volume. Unfortunately this may not be the case as the radiographic joint space is comprised of structures other than articular cartilage [121, 122]. Change in joint space may be affected by other factors such as meniscal extrusion, fluid or even synovitis [121]. In addition, plain radiographs may not portray the joint space accurately. Disease may not uniformly involve all compartments and the associated altered biomechanics may result in an exaggerated joint space despite an involved compartment [124] which is particularly the case if using non-weight bearing images [125].

The use of JSN also suffers from reliability issues [120]. Determining the JSW is highly observer dependent [126]. The observer must determine the narrowest point, this can therefore result in large inter-observer variations [120]. The measurement of JSW is also affected by both the positioning of the knee, and its alignment in respect to the radiograph and source of radiation [16, 115, 127]. Slight variations in joint flexion and/or x-ray beam alignment lead to great variations in the joint space size [16] which becomes problematic when requiring serial measurements.

Finally, while JSN may be used to measure the progression of knee OA [115] it is a crude measure and therefore lacks sensitivity. Only a few studies have demonstrated significant change over short-term periods [128, 129]. This is not surprising given the expected average annual joint space loss is 0.10-0.15mm, however even the most reproducible method can only detect a difference of 0.2mm [119]. JSN also lacks sensitivity because annual rates of cartilage loss are small [130, 131]. Approximately 13% of cartilage volume is lost before the earliest grade of radio graphical OA can be detected [130] and a number of studies have failed to show significant relationships between loss of cartilage and change in JSW [128, 130].

1.6.1.2 Osteophytes

Osteophytes or bony outgrowths are considered the earliest radiographic sign of OA, often being seen prior to JSN [132]. While the grading of OA relies heavily on their presence, their role remains unclear. Osteophytes have not been found to be associated with disease progression [133-135]. For example, among patients with osteophytes within the tibiofemoral compartment fewer than two-thirds developed joint space narrowing in the respective compartments over a 14 year follow up [135]. Studies examining individual radiographic features of OA have also tended to predominantly report results related to the assessment of osteophytes since measures of osteophytes tend to yield better reproducibility than measurements of joint space narrowing (JSN) [136]. This focus on the presence of osteophytes in epidemiological studies may therefore underestimate the role of JSN [137-139]. In addition their relationship to pain symptoms is mixed [39, 135, 140, 141], although related to the presence of pain, osteophytes are not related to the severity of pain [39]. Thus grading systems may lack complete content validity [19].

It is therefore clear that more sensitive methods to image the knee joint are required if the pathogenesis of knee OA is to be fully explored.

1.6.2 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) offers several advantages over radiography. Unlike radiographs MRI allows direct visualization of all components of the joint simultaneously [116, 142]. It offers excellent soft tissue contrast and multi-planar imaging [124, 143]. In addition both quantitative and morphometric analysis of cartilage can be performed [144, 145], as can semi-quantitative whole joint analysis [117, 146, 147]. Methods have been developed to assess many properties of articular cartilage using MRI ranging from thickness and volume, as well as detecting and classifying surface irregularities known as cartilage defects. Other structural changes in the knee including bone expansion, bone marrow lesions, meniscal pathology, synovium, ligaments and any other periarticular features can also be identified. Due to the three-dimensional nature of MRI it does not suffer from the reproducibility issues related to repositioning that radiography does [119]. Finally, MRI does not involve the use of radiation which increases its appeal in research settings. However, MRI is not without its limitations. These include the high cost when compared to radiography, the length of time required to obtain images and contraindications (e.g. pacemakes).

While cartilage has been the predominant focus of studies examining knee OA, other joint structures are involved in the pathogenesis of the disease. MRI allows us to examine other intra and extraarticular structures. The assessment of articular cartilage volume in isolation is unlikely to be the best available means of determining the severity of OA: use of a combination of measures of multiple joint structures associated with disease (eg bone, meniscus, etc) may help to better assess the severity of knee OA.

1.6.2.1 Cartilage Volume

Loss of cartilage volume is considered the hallmark of OA. MRI is able to image articular cartilage better than any other imaging techniques. Knee cartilage volume measured by MRI has been shown to be valid, reliable and reproducible [118, 143, 144, 148-150] and correlates with radiographic grade of OA [130]. By the time the first changes of radiological OA can be detected, 13% of knee cartilage has already been lost [130]. Measurement of knee cartilage volume is sensitive to change in both normal subjects [151, 152] and those with OA [142], where loss of tibial cartilage correlates with worsening of symptoms [153] and predicts knee replacement [38]. MRI has provided the first opportunity to examine knee articular cartilage non-invasively prior to the development of OA, as well as after OA has been established. With this method, the state of knee cartilage can be examined as a continuous variable from the normal to the pre-diseased and then to the early diseased state.

However, the use of cartilage volume in the measurement of OA does not provide any information about the quality of cartilage present. This has been raised as a significant concern in longitudinal studies, since theoretically, and in animal models, cartilage change in OA has been shown to be biphasic, with swelling occurring prior to cartilage thinning and loss [154, 155].

1.6.2.2 Cartilage Thickness

Measuring articular cartilage thickness via MRI has been examined as a quantitative alternative to the radiological assessment of the severity of OA, as well as to identify risk for disease onset and progression [156, 157]. However, several limitations have been identified when adopting cartilage thickness as a means for assessing knee OA. In particular, diurnal variability in articular cartilage thickness, but not volume, has been demonstrated [157]. Additionally, longitudinal studies examining change in cartilage thickness may be limited by the difficulty in reselecting identical section locations at follow-up assessment [158].

1.6.2.3 Cartilage Defects

While a reduction in cartilage volume is a recognised feature of degenerative change, other earlier cartilage lesions, often referred to as cartilage defects, are also apparent prior to radiographic change. Cartilage defects are irregularities on the surface of the usually smooth articular cartilage that can be detected and assessed with MRI [159, 160]. Among asymptomatic individuals there is growing evidence that the presence of cartilage defects may represent early OA [161, 162]. The presence of cartilage defects in asymptomatic subjects has been associated with a reduction in cartilage volume [161, 163].

A number of semi-quantitative scoring methods have been developed to assess cartilage defects. These methods employ a scoring system that grades the severity incorporating both size and depth of lesions and have been shown to be both sensitive and highly specific (reviewed in [164, 165]).

Difficulties in assessing cartilage morphology occur however due to cartilage's thinness and geometrically complex morphology. Changes such as surface fibrillisation and the appearance of repair tissue and osteophytes can also be problematic [164]. There is also the potential for various artefacts to be present in the images, particularly interface between cartilage and bone. In addition, signal changes associated with OA or between baseline and follow up scans can hamper the measurement of cartilage defects. Nonetheless with the appropriate MRI sequences articular cartilage defects can be accurately detected and graded with high sensitivity and specificity [166, 167].

1.6.2.4 Subchondral Bone

Subchondral bone has been recognised as important in terms of the pain and progression of OA [168]. The use of MRI has now allowed us to examine change in subchondral bone that was not previously available from radiographs. MRI studies have shown that people with knee OA have a larger bone surface area at the tibial plateau than healthy controls, and that the rate of bone expansion is greater in osteoarthritis than in healthy subjects [169].

Bone marrow lesions (BMLs) are another common abnormality described in OA. They appear as ill-defined areas of increased signal intensity adjacent to sub-cortical bone on T2 or proton density images or as low intensity when compared to normal bone on T1-weighted images [170]. Both semi-quantitative and quantitative methods have been applied to measure and score the size of BMLs [41, 43, 171-177]. BMLs are associated with knee pain [41, 172, 174-177] and disease progression [78, 175, 178]. Histologically BMLs represent areas of osteonecrosis, oedema, fibrosis, trabecular abnormalities and bony remodeling [170] yet the exact composition of BMLs from MRI cannot be discerned.

1.6.2.5 Meniscal Tears

Meniscal tears are highly prevalent in OA [179-182] and are related to both cartilage changes and symptoms [43, 179, 182-185]. MRI remains the best non-invasive test for assessing meniscal pathology and is both highly sensitive and specific [186]. The sensitivity and specificity of detecting meniscal tear by MRI are both in the range of 82-96% [187, 188]. A common system for grading meniscal damage relates to the distribution of MRI signal intensity and its relation to the articular surface [180] and has been shown to correlate will with histological findings [188]. Tears can be classified as either traumatic or degenerative [81]. With a traumatic tear the meniscus often splits vertically and parallel (longitudinal tear) or occasionally perpendicular (radial tear) to the circumferentially orientated collagen fibres [189-192]. In contrast, degenerative tears include horizontal cleavages, flap tears or complex tears, meniscal maceration or destruction[85].

1.6.2.6 Ligamentous Damage

Studies of anterior cruciate ligament ruptures (ACL) have highlighted the role of knee ligaments in OA [81]. MRI evaluation of ligaments has been shown to be highly sensitive and specific when compared with arthroscopy [193]. ACL and posterior cruciate ligaments are best imaged from sagittal sequences while coronal sequences are best for imaging collateral ligaments [194]. There are a few methods described for the scoring of ligaments from MRI. These involve assessing the grade of injury [195]

i.e a complete tear or not, via assessment of oedema on the sides of ligaments and ligament disruption [196] or if the presence of repair is noted [146].

1.6.2.7 Synovial Changes and Joint Effusion

Inflammation is important in OA [197]. Synovitis and synovial volume changes can be observed by MRI [198, 199]. Synovitis has been shown to play a role in the progression of cartilage loss [198, 200-202] as well as symptoms [203]. Gadolinium enhanced MRI is probably the best way to assess synovial changes [204] however, the use of gadolinium has implications for the subject, duration of scan and selection of sequences. A reasonably good consensus between gadolinium and non-gadolinium scoring can be achieved [204, 205] however it was demonstrated that only the synovial changes detected using contrast enhanced MRI correlated with microscopically proven synovitis [119]. Synovitis can be quantified by either using volume estimates or assessing characteristics of the gadolinium signal with enhanced MRI techniques [194]. Volume can also be assessed semi-quantitatively [119].

1.6.3 Semi-quantitative Whole Joint Assessment

As MRI allows the assessment of the entire joint, and while each of the individual structures can be quantitatively assessed, tools to assess the whole joint have been developed and validated. Semi-quantitative assessment of the joint from MRI allows multiple features to be assessed simultaneously. The system incorporates scores of articular cartilage integrity, sub articular bone marrow abnormalities, subchondral cysts, sub articular bone attrition and osteophytes, meniscal integrity, ligaments, synovitis and effusion, intra-articular loose bodies, periarticular cysts and bursitis [206].

Three scoring systems have been described: the Whole-Organ Magnetic Resonance Imaging Score (WORMS) [117], the Knee Osteoarthritis Scoring System (KOSS) [147] and the Boston Leeds Osteoarthritis Knee Score (BLOKS) [146]. While the scoring methods show adequate reliability, specificity, and sensitivity (discussed in [206]), they are limited by the time needed to assess the images, the number of sequences and individual images required (not all features can be measured from the

same images) and the level of skill in reading the images and documentation of the data [206]. There is also a trade off in image quality as it requires a protocol that allows visualisation of many components simultaneously rather than optimised for each individual component [194, 207]. A result of this is that quantitative approaches have been shown to be more powerful then semi-quantitative approaches in detecting relationships between measures of cartilage volume and local risk factors (meniscal damage and mal-alignment) [194]. However, semi-quantitative methods are still in their infancy and more work is required.

1.6.4 Imaging the Knee Joint: Where to Next?

The ideal imaging modality for the progression and assessment of OA would provide data pertaining to all joint structures, including a direct measure of both cartilage and bone, as well as other intra and extra-articular structures in three dimensions. The ideal measure would be non-invasive, readily available, cheap, valid, reliable, relate to clinical outcomes, and be sensitive to change without exposing a subject to unnecessary ionizing radiation. MRI promises to fulfil many of these criteria.

1.7 Structural Changes in the Knee

The application of MRI assessment of cartilage and other periarticular structures has led to the development of image biomarkers for disease, as well as the identification of risk factors for changes in these structures thereby allowing a further understanding of the pathophysiology of disease. These structures include cartilage volume, cartilage defects, meniscal tears, subchondral bone expansion and bone marrow lesions and are discussed in further detail below.

1.7.1 Cartilage Volume

The loss of articular cartilage volume is considered the hallmark of disease. Approximately 13% of cartilage volume is lost for a knee to progress from a healthy to a diseased joint [130] and by the final stages of disease ~60% of cartilage is lost [142]. Once knee OA is established, cartilage is lost more rapidly than in healthy

adults. In a cohort of patients with symptomatic knee OA, the annual rate of cartilage loss was shown to be between 4.4% and 6.2% over 2 years [142] and 3.1% and 4.8% over 4.5 years [208]. In contrast, the annual rate of cartilage loss among asymptomatic healthy middle-aged women was shown to be 2.3-2.4% [152] and ~2.8% in a small cohort of healthy men, both over 2 years [151]. Furthermore it has also been suggested that cartilage loss is episodic with variability in cartilage loss between those with progressive and non-progressive OA [209].

The relationship between cartilage volume and knee pain is unclear. Although articular cartilage may be viewed as a major target tissue of OA, it is not innervated by nociceptors and as such, cannot be a direct source of pain [210]. Studies using cartilage volume measured by MRI have demonstrated a weak association between cartilage loss and pain [211]. In a recent longitudinal study of 117 subjects with knee OA, a weak association between tibial cartilage volume and symptoms at baseline was seen over 2 years. The severity of the symptoms of knee OA at baseline did not predict subsequent tibial cartilage loss. However, there was a weak association between loss of tibial cartilage and worsening of symptoms. This study suggested that although cartilage is not a major determinant of symptoms in knee OA it does relate to symptoms [211].

Regardless of the relationship cartilage loss shares with symptoms, it is the loss of cartilage that ultimately leads to patients requiring joint replacement. Identifying risk factors that predict cartilage loss is therefore important.

1.7.1.1 Risk Factors for Cartilage Loss

A number of risk factors associated with the loss of articular cartilage have been identified. These include age, female gender, increased body mass index, body composition, hormonal status, smoking, physical activity and knee alignment and are discussed below.

Age

The cross-sectional relationship between age and cartilage volume has been shown to be inconsistent. Cross-sectionally knee cartilage has been noted to be generally

thinner in elderly subjects than in younger subjects [212]. In a small study of healthy men, increased age was associated with reduced medial and lateral tibial and patella cartilage volume [213]. A cross-sectional study of 372 middle-aged subjects suggested an association between increasing age and decreased knee cartilage thickness at all sites but did not show any associations with cartilage volume [214]. It has been suggested that these inconsistent findings may be attributed to an inability of the knee cartilage volume assessment to differentiate normal and swollen cartilage [215]. Findings from longitudinal studies have also reported inconsistent associations between age and cartilage loss [128, 151, 208, 215]. In a predominantly OA free population (only 17% with ROA) cartilage volume was shown to decline at a faster rate with increasing age [215]. However, while age was found to predict loss of lateral cartilage volume over 2 years in symptomatic individuals no relationship was seen when they were followed up over 4.5 years [208]. Increasing age was also not found to be associated with rate of cartilage loss in healthy men over 2 years [151]. Furthermore a study examining predictors of fast and slow progression of knee OA also failed to detect any associations with age [128]. The underlying reason for these differences may reflect variations in sex hormones, growth factors, genetics and other factors.

Gender

Studies have been consistent in their findings that men have more articular cartilage than women, in both adults and children [118, 216, 217]. However, findings from longitudinal studies have shown that women have an increased rate of cartilage loss compared to men. In a community based cohort of asymptomatic adults women lost tibial cartilage at 4 times the rate and patella cartilage at 3 times the rate of men over ~2 years [218]. Similar results have been reported in a predominantly OA free population where women lost substantially more cartilage over 2 years compared to men [215]. Furthermore it was found in a small cohort of asymptomatic subjects that the age related associations seen with cartilage volume were gender dependant with reduced patella thickness being related to increased age in women but not men [212].

Body Mass Index and Obesity

An increased body mass index or obesity has been shown to be associated with reduced cartilage volume cross-sectionally. In healthy men total, lateral and medial

cartilage volumes were inversely associated with BMI [213]. BMI was also negatively associated with cartilage volume in a cross-sectional study of 190 older adults [219]. Findings from longitudinal studies have yielded conflicting results. BMI was not found to be associated with cartilage loss over two years in a cohort of asymptomatic post menopausal women [152] or in a smaller cohort of healthy men [151] however, both groups were predominantly of normal weight. Among participants with symptomatic knee OA, a trend for increased BMI and increased annual percentage loss of cartilage over 2 years was observed [142], this was no longer significant when participants were follow up over 4.5 year [208]. In contrast, BMI predicted cartilage loss among 107 subjects with knee OA over 2 years [220] and in subjects with symptomatic knee OA, a trend for increased rate of cartilage loss and obesity among subjects with ROA was observed [131]. Furthermore in a smaller study of 32 patients with symptomatic knee OA a trend for fast progressors having a higher BMI compared to slow progressors was observed [128]. It has been suggested that the relationship BMI shares with cartilage volume may not be solely biomechanical. For example leptin, a protein encoded on the obesity gene, was found to explain the relationship BMI shared with cartilage volume in older adults inferring that the relationship obesity has with cartilage loss may be metabolically mediated [219].

Body Composition

Although obesity is widely accepted as a risk factor for knee osteoarthritis, there is evidence to suggest that the specific components of weight and body composition are determinants of properties of articular knee cartilage [221-224]. The measure of BMI does not discriminate adipose from non-adipose body mass [225] and may explain the inconsistent associations between BMI and cartilage. In contrast to BMI per se, there is evidence that muscle mass protects the knee joint while fat mass may be detrimental [221-223]. Findings from a cross-sectional study of 297 asymptomatic community based adults demonstrated that increased fat-free mass was positively associated with tibial cartilage volume while fat mass was negatively associated with tibial cartilage volume[223]. Increased muscle mass was also associated with increased tibial cartilage volume cross-sectionally and reduced cartilage loss over 2 years in healthy subjects however no association with fat mass and cartilage was observed [224].

Hormonal Status.

Oestrogen receptors are present on human articular chondrocytes and there is experimental evidence to suggest that oestrogen has a physiological role in the normal joint [59]. However, there is limited epidemiological data examining the role of hormones on cartilage volume. A cross-sectional study suggested that postmenopausal women using long term (longer than 5 years) oestrogen replacement therapy (ERT) had more tibial cartilage than controls [226]. When the same cohort was followed longitudinally for 2.5 years, ERT was not associated with the rate of reduction of tibial cartilage volume [152]. In a small group of 45 healthy men, there was a positive association between serum testosterone and tibial cartilage volume at all tibial cartilage sites, but only reached statistical significance for medial tibial cartilage [213].

Smoking

Cigarette smoking has been shown to be associated with an increased loss of knee cartilage in cohorts mixed in relation to OA diagnosis and/or symptoms [227-229]. In a cohort largely without radiographic knee OA, smoking and pack-years smoked was associated with increased medial and lateral femoral cartilage loss over approximately 2 years [227]. In a 30 month follow up of 159 men with symptomatic knee OA, smokers were found to lose more medial tibiofemoral and patella cartilage [228]. More recently, in a population mixed in relation to OA diagnosis, smoking was found to be associated with increased cartilage loss primarily in people with a family history of knee OA over 2.5 years [229]. Despite these consistent findings the mechanism by which smoking affects cartilage remains unclear.

Physical Activity

Cross-sectional and longitudinal data in children suggests that cartilage growth responds to stimulation from exercise [217, 230]. Children who exercised more had higher cartilage volumes than those who did not [217]. Longitudinal data has suggested that this may be due to exercise level, with those who participated regularly in sport, above the median, gaining more tibial cartilage than those who were below the median [230].

Knee Alignment

Changes in knee alignment may redistribute the medial and/or lateral loads at the joint, potentially leading to cartilage damage. Studies that have examined the relationship between joint alignment and cartilage volume have consistently shown a relationship between alterations in joint alignment and reduced cartilage volume [76, 231]. Among 117 subjects with knee OA, a more valgus knee angle was associated with less tibial cartilage within the lateral compartment cross-sectionally [231]. When these subjects were examined longitudinally over 2 years a more varus knee at baseline was associated with increased medial cartilage loss, while a more valgus knee with lateral cartilage loss [231]. Similarly, in 153 people with knee OA varus knee alignment predicted medial tibial cartilage volume and thickness loss over 2 years [232]. Among 174 subjects with symptomatic knee OA, varus alignment was also associated with increased loss of medial tibial cartilage volume and thickness compared to neutral knees and valgus alignment was associated with more lateral cartilage loss over 2 years [131].

Knee Structural Changes

A number of structural changes in the knee have also been associated with cartilage loss and subsequent knee OA development and/or progression. These include cartilage defects, meniscal tears, subchondral bone expansion and bone marrow lesions and are discussed further in the following sections.

1.7.2 Cartilage Defects

Defects in articular cartilage have aroused particular interest. They are found in healthy subjects as well as in those with OA [233, 234] and are shown to have a fluctuating course [235]. Cartilage defects have been shown to be significantly associated with radiographic and clinical features [137, 140, 175, 181, 236, 237], predict knee cartilage loss [161, 163] and knee joint replacement [237]. It is thought that cartilage defects may play an important role in early OA [163] and subsequently lead to cartilage loss and OA. Interventions aimed at reducing or reversing cartilage defects may reduce the risk of subsequent knee OA.

1.7.2.1 Cartilage Defects in OA Populations

In OA populations cartilage defects are extremely common. Cartilage defects were present in 81% of medial and 64% of lateral tibiofemoral compartments and 55% of patellar cartilages of 117 subjects with symptomatic knee OA [237]. In 50 patients with varying stages of disease, cartilage defects were present in 43/50 knees [181]. Similarly, cartilage defects were identified in 61% of knees of patients with symptomatic knees undergoing arthroscopic surgery [234].

The natural history of cartilage defects in osteoarthritic populations is largely unknown. In a small study of 43 patients 38% of mild (grade 1) defects progressed, while 23% of mild lesions improved over 1.8 years [182].

1.7.2.2 Cartilage Defects in non-OA Populations

Cartilage defects are also common in non-OA populations, however they have been found to be less severe than seen in OA populations. In 124 healthy adults, 35% of knees had medial and 48% had lateral cartilage defects of which 47% were classified as grade 1 lesions [238]. In 86 healthy men and women, 61% had medial and 43% had lateral cartilage defects, however grades were also low [163]. Among 372 predominantly non-OA subjects, cartilage defects were present in 44% of people and varied in severity from grade 2-4 [233].

Studies examining the natural history of cartilage defects in non-OA populations have found that less severe defects are likely to progress, while those of greatest severity are likely to improve [235, 238]. In 124 healthy adults followed over 2 years, 63% of defects in the medial and 65% of defects in the lateral tibiofemoral compartment got worse. Less severe cartilage defects were more likely to progress, while those that were more severe were more likely to improve slightly [238]. In a predominantly OA-free population where the majority of people have less severe defects, 33% of subjects had a worsening defect score while 37% had improvement over ~2 years [235]. These results suggest that knee cartilage defects are reversible and may represent and an intermediate factor to study early in the natural history of knee OA.

1.7.2.3 Significance of Cartilage Defects

The presence of cartilage defects is associated with severity of radiographic disease including KL score [140, 175] and osteophytes [137, 140, 181] as well as knee symptoms [239] and joint replacement [237]. Among 372 predominantly non-OA subjects, both the prevalence and severity of cartilage defects was associated with JSN and osteophytes [233]. Similarly in small study examining patients with varying stages of disease, cartilage defects were associated with KL grade [181]. In another small study of 59 people with knee pain, cartilage defects correlated with the presence of osteophytes [140]. Higher cartilage defect scores were also associated with a 6-fold increased risk of joint replacement over 4 years compared to those with less severe defects in subjects with symptomatic knee OA [237].

Cartilage defects are also associated with reduced cartilage volume cross-sectionally [233] and predict loss of cartilage over time [161, 163, 233, 237]. In a large cross-sectional study of predominantly OA free individuals, cartilage defects were associated with reduced cartilage volume [233]. In 86 healthy men and women, subjects with cartilage defects had a 25% reduction in medial tibial cartilage volume and a 15% reduction in lateral volume relative to those with no defects. Furthermore, those with medial defects had 1.9 times increased loss of medial cartilage volume compared to those who did not over 2 years [163]. Similarly in subjects with symptomatic knee OA, those with patella cartilage defects had a higher rate of annual cartilage loss compared to those who did not [237].

The relationship between cartilage defects and symptoms is unclear. This is not unexpected given that cartilage does not contain pain fibres. In subjects with symptomatic knee OA, cartilage defects were not found to be associated with pain or stiffness [43]. In contrast however in another small study comparing different stages of disease most knee symptoms were reported in knees with grade IIa lesions(<50% cartilage loss) [181]. Link and colleagues hypothesised that clinical symptoms may be more substantial at the onset of OA, particularly as no association with more severe defects and pain was seen [181]. While the presence of defects alone has not been linked to pain, full thickness defects in the presence of subchondral bone changes have [175].

1.7.2.4 Risk Factors for Cartilage Defects

The prevalence and severity of cartilage defects has been shown to increase with increasing age [214], female gender [162, 214], increased BMI [162], due to genetic factors [240] and with increased tibial plateau area [237]. Among predominantly non-OA participants, increased age, BMI and tibial plateau area were associated with the increased prevalence and severity of cartilage defects, and the effects of both age and obesity were stronger in women compared to men [162, 214]. In a population based case-control sib-pair study, subjects with a family history of knee OA had a higher prevalence of cartilage defects compared to controls. In the sib-pair component, knee cartilage defects were also shown to have a high heritability for both scores and prevalence [240]. In asymptomatic knees, cartilage defects have also been have been attributed to trauma [182].

Studies examining the natural history of cartilage defects have found in a healthy population, that worsening of defects is associated with increasing age and male gender [238]. In a predominantly OA-free population, progression of defects was associated with female gender, increased age and BMI and tibial plateau area [235]. Cartilage defects also had a higher rate of progression in women compared to men in healthy asymptomatic subjects followed over ~2 years [218].

1.7.3 Meniscal Tears

Meniscal tears have been identified as an important risk factor for the development and progression of knee OA [180]. The menisci are two semicircular fibro-cartilaginous disks covering approximately two thirds of the corresponding articular cartilage. They are located between the medial and lateral articular surfaces of the femur and tibia [241]. While the menisci play a role in stability enhancement and lubrication [241, 242], their most important functions are as load bearers and shock absorbers. The menisci transmit anywhere from 45-60% of the compressive loads of the knee [83]. When a tear occurs, the menisci are not longer able to resist axial loading. This results in increased peak and average contact stresses on the cartilage. For example within the medial compartment a 40-700% increase in contact stresses can occur in the presence of a medial meniscal tear [243-245]. It is thought that this

increase in contact stress to articular cartilage and subchondral bone may initiate the development of OA with early degenerative meniscal damage being a signal of early osteoarthritic disease.

1.7.3.1 Meniscal Tears in OA Populations

Meniscal tears have predominantly been identified in osteoarthritic populations where they have been shown to be highly prevalent and more common within the medial compartment. In people with symptomatic and/or radiographic knee OA the prevalence of meniscal tears has been found to be ~63%-91% [179-182]. Among elderly participants of the Boston Knee Osteoarthritis study (BOKS) 91% of participants had a meniscal tear [179]. Similarly, among middle-aged and elderly people within the Framingham cohort, 63% of those with symptomatic ROA, and 60% who had asymptomatic ROA had a meniscal tear [180]. Similar findings have been found in a smaller studies [181, 182]. Meniscal tears are also prevalent in populations with knee pain; tears were present in 67% of knees in a mixed population with knee pain (only 47% with ROA) [43]. Among participants of the Multi-centre Osteoarthritis Study (MOST) who either have or are at high risk of OA, 38% of symptomatic knees had a meniscal tear [183].

1.7.3.2 Meniscal Tears in Non-OA Populations

There are very limited data about the prevalence of tears in asymptomatic or non-OA populations; nonetheless they have been shown to also be common within these populations. Among a sub-group of the Framingham cohort who had no evidence of ROA or symptoms, tears were present in 23% of people [180]. A much higher prevalence was reported among asymptomatic controls of the BOKS study where 76% of people had a meniscal tear [246] a similarly high prevalence was reported among a predominantly non-OA population with 72% of people having tears [184]. However the slightly higher prevalence's reported in these two studies may be attributed to a small percentage either having early radiographic knee OA or because they are at high risk of developing OA.

1.7.3.3 Significance of Meniscal Tears

They presence of a meniscal tear is significantly associated with both the presence and severity of radiographic features of OA [83, 184, 247]. A cross-sectional study of people at high risk of developing knee OA found that meniscal tears were associated with increased JSN and presence of osteophytes [184]. Similar findings were seen in another cross-sectional study that found that meniscal subluxation correlated with severity of JSN [247]. In a longitudinal study of patients who had undergone meniscectomy, meniscal tears were also associated with both radiographic and combined symptomatic and radiographic OA over 16 years [83].

Meniscal tears have also been shown to be associated with structural changes within the knee assessed by MRI [179, 182, 184, 185]. Results from a cross-sectional study of a predominantly non-OA population, found that meniscal tears were associated with reduced cartilage volume and increased cartilage defects [184]. This has been confirmed by longitudinal studies [179, 185]. A higher rate of cartilage loss assessed semi-quantitatively was observed among the elderly participants with symptomatic knee OA [179]. In addition. a stronger effect was observed within the medial compared to lateral compartment [179]. Amongst people with symptomatic knee OA, highly significant differences in global and medial cartilage loss between severe medial meniscal tears and absence of tears was demonstrated with severe meniscal tears predicting faster disease progression over 2 years [185]. Similar results were seen in a smaller cohort of 43 patients undergoing knee MRI. Those who had a meniscal tear showed a higher average rate of progression of cartilage loss (assessed semi-quantitatively) compared to those without a tear[182].

1.7.3.4 Risk factors for Meniscal Tears

In younger, active adults meniscal tears are more likely to have occurred due to trauma to an otherwise healthy knee, whereas tears observed in middle-aged persons are likely to be the results of degenerative processes [85]. It is not surprising then that risk factors for tears include age, gender, increasing body mass index and a family history of knee OA [180, 184, 246]. Among elderly participants of the Framingham cohort, age and male gender were associated with increased prevalence of tears.

Among women, BMI was also associated with increased tears [180]. Male gender was also shown to be associated with meniscal tears amongst participants of the BOKS study [246]. Similar results were seen in a predominantly non-OA population where participants with a meniscal tear were significantly older and had a higher BMI [184]. In addition, those participants who had a family history of knee OA had higher odds of tear at the medial and lateral anterior and posterior horns compared to those that did not have a family history [184]. In contrast, within this population women were found to have slightly higher odds of medial or lateral body tears compared to men. Considering women have a greater risk of OA, this may not be surprising. As most research has been performed in populations who have undergone meniscectomy or have knee OA further research is required to identify possible modifiable risk factors for meniscal tears in non-osteoarthritic populations.

1.7.4 Subchondral Bone

While articular cartilage has been the primary focus of researchers, bone changes have also been proposed to be an important element in the pathogenesis of OA [168]. It has been suggested that the changes in subchondral bone may be the initiating factor in the pathogenesis of OA rather than the sequelae of cartilage damage. However, it remains unclear and controversial whether OA is initiated in bone or cartilage. In adults, subchondral bone area is not static. Tibial plateau bone area was found to increase by 0.8–1.2% per annum over 2.5 years in healthy women [248]. In subjects with knee OA the medial and lateral tibial bone areas increased by 2.2% and 1.5% per annum, respectively, over 2 years [249]. These changes affect the mechanical properties of the subchondral bone and may reflect the changes in its architecture. The enlargement of the bone area of the tibial plateau may attenuate the tibial cartilage, and this attenuation may play a role in the process of OA.

1.7.4.1 Subchondral Bone Expansion and OA

A relationship between increased bone size and radiographic OA has been established [169, 250]. Subjects with OA have larger bones than healthy controls [169, 251]. In a cross-sectional study comparing women with clinically diagnosed OA and those without, it was found that women with knee OA had larger tibial plateau bone sizes

than women with healthy knees [169]. Tibial plateau expansion has also been shown to be associated with the radiographic features of OA [169, 250]. In a cross-sectional study of women with knee OA, a larger tibial plateau area was associated with increased JSN and osteophytes. In addition, for each increase in grade of osteophyte and joint space narrowing, bone area in the medial and lateral sites increased substantially [169]. Similar results were observed in predominantly non-osteoarthritic population [250].

Tibial plateau expansion has been shown to be associated with prevalence and severity of knee cartilage defects [233] and also with worsening of tibiofemoral defects over 2 years [235]. Given that cartilage defects have been shown to predict cartilage loss, this suggests that subchondral bone changes precede cartilage damage in early knee OA. Furthermore, while bone expansion has not been found to predict cartilage loss, bone changes are predictive of total knee replacement due to severe knee OA. Amongst subjects with symptomatic knee OA, tibial subchondral bone area at baseline was an independent predictors of knee replacement over 4 years [38].

1.7.4.2 Factors Affecting Bone Expansion

A number of risk factors for OA are also associated with tibial plateau expansion. These include increasing age, male gender [162, 214, 248], body mass index [162, 249], increased adductor moment [252-255], genetic factors [256, 257], and physical activity [257].

Age

Bone area increases with age [169, 214, 258-262]. Findings from cross-sectional studies have demonstrated that age was positively associated with an increased tibial plateau area [214, 259, 262]. While the reason for the increase in cross-sectional bone area in older age remains unknown, it has been hypothesised that the increase could be regarded as a compensatory mechanism to maintain bone strength, amidst a decrease in bone mineral density that also occurs in aging [259, 262].

Gender

Men have larger tibial bone sizes than women [260]. Similar results have been reported by others in both cross-sectional and longitudinal studies [118, 249]. Furthermore tibial plateau bone area was shown to increase with age at a faster rate in men than in women [260]. These findings are discordant with epidemiological data of OA, which indicates that before the age of 45, men have a higher prevalence of OA than women, and from then on it is women who have the higher prevalence [263]. Thus it appears, particularly in women that factors other than gender must be important in the pathogenesis of OA. Gender does affect bone size, but may also be associated with some other aspect of bone; the current research in the field does not offer any definitive answers on this issue, but so far implicates the obvious postmenopausal alterations in the female endocrine system [264, 265] .

Body Mass Index

Recent studies have shown that patients with a higher BMI also have a larger tibial bone size [162, 214]. Two cross-sectional studies of predominantly healthy adults demonstrated that BMI was positively associated with an increased tibial plateau area [162, 214]. Similar findings have been reported in a longitudinal study of subjects with knee OA [249]. The relationship between BMI and bone expansion may be due to body habitus being associated with bone size [266], due increased loading stimulating bony expansion or even a metabolic effect [267].

Biomechanics and physical activity

Factors that lead to increased joint loading have been found to correlate with increased tibial bone area. These include not only BMI (discussed above) but also static joint alignment, gait parameters and physical activity. In a longitudinal study of healthy women, a trend for knees becoming more valgus and lateral bone expansion was observed [248]. A greater adduction moment has also been shown to correlate significantly with a larger medial tibial plateau bone area [254]. The knee adductor moment is the natural reaction force of the ground causing adduction of the tibiofemoral joint during gait [268]. It is a major determinant of 70% of the total knee joint load passing through the medial tibiofemoral compartment during walking [252]. Earlier research has also found an association between the increased adductor moment in the medial compartment of the knee joint, and amount of bone distributed to the

medial segment of the tibial plateau [252, 253, 255]. In addition physical activity has also been found to be positively associated with bone expansion in women [269].

Bone size has been shown to be an inherited trait [38, 257]. In a study that compared tibial plateau bone size in healthy subjects with familial history of OA and controls matched for age and sex, those with OA ancestry had larger tibial plateau regions than those who did not [38]. This is supported by the findings of an earlier study that examined the heritability of bone size using a sibling-pair design, and indicated that size of the tibial plateau was under strong genetic influence [257]. These findings suggested that the heritability of tibial plateau size was mediated by body size and that a structural gene or genes may be the reason for the transmission of this trait from parent to offspring [257].

1.7.5 Bone Marrow Lesions

Bone marrow lesions (BMLs) have been implicated in the pathogenesis of knee OA. They have been shown to be present in both symptomatic [41, 181, 270, 271] and asymptomatic populations [175, 272, 273]. Histological examination of BMLs in knees has shown that they may represent areas of osteonecrosis, oedema, trabecular abnormalities and bony remodeling [170]. It is thought that the role BMLs play in the pathogenesis of OA may involve the reduction in strength of the bony foundation of articular cartilage [274, 275] and/or in impairing the supply of nutrients and oxygen to the overlying cartilage plate [274-276].

1.7.5.1 Bone Marrow Lesions in OA Populations

Among patients with OA BMLS are highly prevalent, being observed in ~50-80% of knees [41, 43, 175, 181, 270, 277, 278]. Among community based women mixed in relation to ROA and symptoms, BMLS were seen in 64% of knees [175]. This is consistent with findings in similar cohorts of patients mixed in relation to ROA [181, 279] and in patients with symptomatic ROA of the knee, the prevalence of BMLs has been shown to range from ~50-82% [41, 43, 181, 270, 277, 278].

The incidence of BMLs in cohorts mixed in relation to radiographic OA diagnosis and/or pain symptoms has also been reported [239, 271, 278]. In a population with chronic knee pain, in which 80% had radiographic knee OA, new lesions developed in 21% of people over 2 years [278]. Similarly, amongst patients with primary knee OA, new BMLs developed over 15 or 30 months in 20% of knees [271]. In a small study of 47 people with painful knees with (88%) or without OA (12%), 8 new BMLs were identified over 2 years [239].

In OA populations, once present BMLS are unlikely to resolve. In a longitudinal study of subjects with symptomatic knee OA, 99% of BMLs either remained the same or increased over 15 or 30 months [271]. Another study of middle-aged sibling pairs with symptomatic knee OA found that only 10% of BMLs resolved over 2 years [278]. Similarly, in a population with chronic knee pain, which included subjects with and without radiographic OA, only 22% completely resolved over 2 years [239].

1.7.5.2 Bone Marrow Lesions in Non-OA Populations

More recently BMLS have also been reported in asymptomatic non-OA populations [175, 272, 273]. Within two asymptomatic clinically healthy populations BMLS were found to be present in approximately 13% of people [272, 273]. A higher prevalence of BMLs was seen in community based women free of ROA and pain with 35% of knees having a BML [175]. The incidence and natural history of BMLs in non-OA populations has not been examined.

1.7.5.3 The Significance of Bone Marrow Lesion

The presence of BMLs within the knee has been associated with the severity and progression of radiographic knee OA [78, 175, 178]. Among women aged 35-55 years from the Southeast Michigan (SEM) study, BMLs were more likely to be found in knees with ROA then knees without ROA; 63% of symptomatic ROA knees and 76% of asymptomatic ROA knees had BMLs present compared to only 33% seen in asymptomatic non-OA knees [175]. Furthermore, in a small study of patients with different stages of knee OA, a significant increase in the prevalence and severity of BMLS was seen in knees with severe OA (KL grade of 3 or 4) compared to knees

with mild or no OA [178]. BMLs have also been shown to predict progression of OA [78]. Among participants of the BOKS study, a ~6.5 fold increased odds of joint space narrowing over 15 or 30 months was reported in people with BMLs in their knees. Furthermore, medial progression was predicted by medial lesions and lateral progression by lateral lesions after adjusting for confounders [78].

BMLs are also correlated with individual structural changes within the knee assessed by MRI. Findings from both cross-sectional and longitudinal studies have revealed that BMLs are associated with the prevalence and severity of cartilage defects in both OA [175, 280] and non-OA populations [171, 173]. Amongst patients with mild knee OA (47% with KL of at least 1), BML presence was cross-sectionally associated with the presence of cartilage defects within the same compartment [280]. In patients about to undergo knee surgery, BMLs were seen below cartilage defects identified by arthroscope and the average depth and cross-sectional area of BML increased with increasing cartilage defect grade. For example an overlying cartilage defect with a grade of 2b (at least partial thickness defect greater than 50% or the total thickness of articular cartilage) or higher was found above 87.4% of BMLs [277]. Similarly among women of the SEM study the size of BMLs present correlated with the severity of cartilage defects observed. 47% of knees that had a full thickness defect also had a large BML [175]. Similar results have also been reported in asymptomatic clinically healthy populations [171, 173].

Cross-sectionally BMLs have been shown to be associated with reduced cartilage volume. Among 377 patients with symptomatic ROA, urinary CTX-II (a biomarker of cartilage breakdown) significantly correlated with severity of BML score within the knee. Patients with a BML score >8 had on average, 45% higher CTX-II levels than patients with a score of 0 [270]. BMLs have also been shown to predict cartilage loss. Among patients with symptomatic knee OA, a higher BML score (assessed by the semi-quantitative WORMS method) at baseline predicted increased cartilage loss assessed by MRI over 15 or 30 months [271]. While in patients with symptomatic ROA, worsening of BML score was also associated with increasing urinary CTX-II, over 3 months [270].

Data describing the relationship between BMLs and clinical features of OA is conflicting, although a number of studies report a relationship between BMLs and pain [41, 172, 174-177], others show no such association [43, 181, 270, 278]. Subchondral bone has been suggested as a contributor to knee pain [168] however, the exact causes of knee pain remain unclear, it is known that the marrow of subchondral bone is richly innervated with nociceptive pain fibres [210]. In a cross-sectional study of elderly people with knee OA, the presence of BMLs was associated with knee pain, with larger BMLs being more strongly associated with pain than smaller ones [41]. Among 143 community based individuals with knee OA, BMLs were significantly associated with knee pain and increased BML score was associated with an increased pain score [176]. Similarly in a cross-sectional study of women in the SEM study, larger BMLS (>1cm) were significantly more common in women with painful OA [175]. More recently among subject with or at high risk of knee OA, both the development of new and enlargement of existing BMLs were associated with the development of pain, aching or stiffness over 15 months. In addition, compared to knees that did not develop any symptoms, those that did showed larger BML increases [172]. In contrast no association between BMLs and pain or stiffness was seen among older participants (mixed in relation to OA (39% had symptomatic OA and 38% had a KL score >2)) cross-sectionally [43]. An increase or decrease in BMLs over 2 years was also not associated with severity of WOMAC score [278]. Differences in the results however may be attributed to the latter population having less severe OA. BMLs have also been shown to be related to functional deficits. Among 664 patients with knee complaints including pain, swelling, instability and or knee locking, patients who had a BML in their knee at baseline reported significantly reduced function and decreased activity compared to those who did not [281].

The emerging evidence highlights the important role of BMLs in the pathogenesis of knee OA. Identifying and targeting modifiable risk factors for BMLs may offer another target in the prevention of OA.

1.7.5.4 Risk Factors for Bone Marrow Lesions

A number of risk factors for BMLs have been identified. BMLs were first described in the setting of anterior cruciate ligament injury [282, 283]. Subsequently BMLs have

commonly been described following knee trauma [281, 284-288]. BMLs have also been shown to be associated with biomechanical factors including joint mal-alignment [78] and increased body weight [173]. Amongst elderly participants of the BOKS study who had symptomatic knee OA, a significant relationship between frontal plane alignment and BMLs was observed. Limbs with varus alignment, particularly if marked (≥7°) had a remarkably higher prevalence of medial lesions compared to knees that were valgus or neutral (74.3% v's 16.4%) and limbs with valgus alignment had a higher prevalence of lateral lesions [78]. Among a cohort of clinically healthy asymptomatic middle-aged women, increasing body weight was associated with the prevalence of BMLs [173]. The above suggest that BMLs may be a result of increased loading and is further supported by findings that BMLs are associated with increased local bone density. Among participants of the Framingham cohort the mean medial: lateral (M:L) bone mineral density ratio (which gives an indication of load distribution within the knee joint) was significantly different between knees with and knees without BMLs. Knees with medial BMLs had a higher M:L ratio while those with lateral BMLs had a lower M:L ratio, thus suggesting that BMLs are associated with loading within the joint [174].

BMLs are not solely the consequence of biomechanical factors. More recently systemic factors such as osteo-protective medications [279] and nutritional factors [289, 290] have been shown to be affect the risk of BMLs. In a cross-sectional study of 818 post-menopausal women, bisphoshonate and estrogen (bone anti-resorptive agents used to prevent or treat osteoporosis) use was associated with significantly less BMLs compared to women who had not received these medications. Anti-resorptive users had a 55% reduced odds of having a BML compared to non-users. The mechanism by which anti-resorptive medication may influence the development of BMLs may involve reducing subchondral bone resorption and/or inflammation [279]. Among asymptomatic clinically healthy community based men and women, higher intakes of n-6 polyunsaturated and monounsaturated fatty acids were associated with the increased likelihood of having a BML [291], while a higher intake of vitamin c and fruit was associated with a lower likelihood of having a BML [289]. The above suggest vascular factors affect subchondral bone however further research is needed. Given that subchondral bone is highly vascularised this may represent an area in which preventive strategies for OA may be targeted.

1.8 Evidence That Vascular Pathology May Play a Role in OA

There is growing evidence that vascular disease may be intimately related to the pathogenesis of OA. The prevalence of vascular disease and cardiovascular risk factors is high amongst people with OA [292-294]. Findings from the third NHANES (2002) showed that among people with OA, ~40% had hypertension, 11% had diabetes and 32% had elevated cholesterol (≥240mg/dL) [294].

1.8.1 Cardiovascular Disease and OA Share Common Risk Factors

Emerging evidence suggests that these conditions may share risk factors including elevated cholesterol and triglycerides, hypertension and smoking [28, 294, 295].

Hypercholesterolemia and hypertriglyceridemia

Hypercholesterolemia and hypertriglyceridemia, both risk factors for cardiovascular disease, have been related to the risk and progression of OA [28, 294, 295]. Among patients selected based on hospitalization for joint replacement due to advanced OA, ~38% had hypercholesterolemia (serum cholesterol ≥6.2mmol/L or on antihyperlipidemic medications) [295]. Among women of the Chingford study, moderately raised serum cholesterol levels (6.0-7.1mmol/L) were also associated with the presence of radiological and bilateral knee OA [28]. Hypercholesterolemia and high serum cholesterol levels (3rd versus 1st tertile) were independently associated with generalized OA, and this was almost exclusively due to participants with knee OA [294]. While in the 3rd NHANE survey, 32% of patients with OA had high total cholesterol [294].

Hypertension and Diabetes

Hypertension and diabetes are more prevalent among patients with OA than those without [292, 294]. Findings from the 3rd NHANE survey indicated that 40% of OA patients had hypertension and 11% had diabetes [294]. Hypertension was associated with the risk of knee OA in the Chingford study [296]. Higher systolic blood pressure in 46 patients with either hip or knee OA compared to 23 non-osteoarthritic controls

was also reported in case-control study [297]. Similar findings have been reported in other groups with OA [293, 298].

Smoking.

Smoking affects the risk and progression of knee OA, however results from epidemiological studies are conflicting [53, 58, 64, 108, 299-301]. While some studies have suggested a protective effect of smoking on the risk of knee OA [58, 64, 108, 299], others have reported no such an association [53, 300, 301]. The above studies used radiographic OA or joint replacement as the outcome measure. In contrast studies employing MRI to assess changes in cartilage have yielded different findings. Although a cross-sectional study reported that increased cartilage volume was associated with smoking [302] longitudinal studies have reported that smoking is associated with increased cartilage loss and progression of cartilage defects [227-229]. In men with symptomatic knee OA, smoking was found to be associated with an increased risk of cartilage loss within the medial tibiofemoral and patellofemoral compartments [228]. Smoking was also associated with increased femoral cartilage loss over 2 years in a population with pre-clinical knee OA [227]. In a population mixed in relation to OA diagnosis smoking was also associated with increased cartilage loss and cartilage defect development, primarily in individuals with a family history of knee OA [229].

Dietary lipids

Fatty acids have been implicated in osteoarthritis (OA) [303-305]. Elevated levels of fat and n-6 polyunsaturated fatty acids have been found in OA bone [303] while n-3 polyunsaturated fatty acids have been shown to alleviate progression of OA through an effect on the metabolism of articular cartilage [304, 305]. However, the mechanism by which polyunsaturated fatty acids affect knee structure and consequently the risk of knee OA has not been fully elucidated. Dietary fatty acids associated with an increased risk of cardiovascular disease were also associated with an increased prevalence of bone marrow lesions (BMLs) in a healthy population without clinical knee OA [290].

1.8.2 Vascular Mechanisms in OA

There are a number of mechanisms by which vascular pathology may contribute to the development of OA. The ends of bones are particularly susceptible to vascular insult [306]. Venous occlusion resulting in small vessel stasis underlying the cartilage plate, joint hypertension, hypercoagulability and/or microemboli may all result in subchondral bone ischemia [274]. The resulting disturbances to subchondral bone nutrition and repair may impair the supply of nutrients and oxygen to the overlying cartilage plate [274, 275]. Given, the 'backup' system of nutrient and periosteal arteries is not present at the epiphyseal regions of long bones because of the joint cartilage at this site, the epiphyses and articular surfaces are particularly at risk of circulatory insufficiency. Bone ischemia may also result in osteocyte death, attraction of osteoclasts and excavation of the non-viable bone thereby reducing the strength of the bony foundation of articular cartilage [307]. Repeated episodes of this process at the ends of affected long bones could lead to altered remodelling and bone morphology. In the extreme, there could be partial or total collapse of the subchondral bone, as seen in avascular necrosis.

Increased scintigraphic activity in the joint, which depends on the vascularity of the joint and the turnover of subchondral bone has also been shown to be a strong risk factor for progression of knee OA [307]. This suggests that abnormalities in subchondral bone, possibly driven by vascular disease, are the drivers of progressive joint damage [308]. In support of this, there is recent evidence to suggest that bone marrow lesions are the consequence of ischemia and/or reperfusion injury [275, 309]. A study of human bone marrow utilizing gadolinium demonstrated that compared to knees free of BMLs, those with BMLs showed perfusion abnormalities including a significantly reduced venous outflow [309]. This is perhaps not surprising given that the histology of BML is heterogeneous and includes osteonecrosis, oedema, trabecular abnormalities and bony remodelling [170].

The emerging evidence supports the notion that OA has multiple aetiologies, which converge to produce the recognized manifestations of joint pain and stiffness and degeneration of articular cartilage at the end stage joint. However, a lack of understanding of the underlying cause(s) for OA has meant that treatments remain

largely palliative, with joint replacement an option in end-stage disease. Exploring the possible associations' vascular pathology has with structural changes in the knee associated with disease development and progression has the potential to provide a novel approach to understanding the pathogenesis of knee OA, with implications for the prevention and early treatment of disease.

1.9 Importance of Studying Populations with and without OA

The use of MRI to the study of knee OA has begun to change the way OA may be viewed. MRI allows the construct of OA to be examined as a continuum: as the healthy knee develops progressive structural changes, a gradual metamorphosis occurs from the healthy knee to one with established OA. How a certain risk factor or structural element behaves in osteoarthritic populations may be different to how it does in healthy populations. Given that there is no cure for OA or treatment that slows the progression understanding the interactions in different population types will allow a better understanding of the pathogenesis of knee OA while also allowing preventative methods to be appropriately targeted.

1.10 Aims of This Thesis

This thesis examines the effect of biomechanical and systemic risk factors on knee cartilage and bone and their change over time in both symptomatic/healthy subjects and in those with knee OA.

More specifically this thesis examined:

1. The natural history of cartilage defects in subjects with knee OA
2. The prevalence and significance of meniscal tears and also the relationship between gait parameters and meniscal tears in a cohort of asymptomatic women.
3. The effect of biomechanical factors at the tibiofemoral and patellofemoral compartments in subjects with knee OA
4. The natural history and significance of bone marrow lesions in those with no clinical knee OA.

5. The relationship between systemic/vascular factors including smoking, dietary fatty acids and serum lipids and knee structure in asymptomatic participants.

The results of this study provide a better understanding of the pathogenesis of knee OA with implications for both the prevention and early treatment of disease.

Chapter 2: Populations and Methodology

2.1 Study Populations

This thesis is comprised of 10 studies based on a 4 different study populations. Each population is presented in detail below.

2.1.1 Group 1 - Patients with Established Knee Osteoarthritis

One hundred and thirty-six patients aged over 40 years who fulfilled ACR clinical and radiographic criteria for knee OA [1] with osteophytes present within the knee were recruited by using a combined strategy including advertising through local newspapers and the Victorian branch of the Arthritis Foundation of Australia, as well as collaboration with general practitioners, rheumatologists and orthopaedic surgeons. Subjects were excluded if any other form of arthritis was present, if there was any contraindication to MRI, if a total knee replacement was planned, or if they were unable to cooperate with study requirements. The study was approved by the Ethics Committee of the Alfred and Caulfield hospitals in Melbourne, Australia. All patients gave written informed consent.

2.1.2 Group 2 –Healthy Asymptomatic Post-menopausal Women

Eighty-one postmenopausal women aged over 50 years, who were either current users of ERT (estrogen replacement therapy) (for five or more years) or had never used it (controls), matched by age (± five years) and years since menopause (as defined by the subjects; ± five years), were recruited through the Jean Hailes Centre, private consulting clinics, and advertising in the local media. The exclusion criteria were: inflammatory arthritis, previous knee joint replacement, malignancy, fracture in the last 10 years, and contraindication to MRI (pacemaker, history of potentially ferromagnetic material in a strategic location for example, orbit) [226]. The study was approved by the Alfred Hospital, Caulfield Hospital and La Trobe University ethics committees. All patients gave written informed consent.

2.1.3 Group 3 - Healthy Community-Based Men and Women

The Melbourne Collaborative Cohort Study (MCCS) is a prospective cohort study of 41,528 community-based people, aged 40-69 years at recruitment which occurred between 1990 and 1994, with the aim of examining the role of lifestyle and genetic factors in the risk of cancer and chronic diseases [310]. Participants for this current study were recruited from MCCS. As the aim of the current study was to investigate subjects with no significant current or past knee disease, individuals were excluded if they had had any of the following: knee pain lasting for > 24 hours in the last 5 years; a previous knee injury requiring non-weight bearing treatment for > 24 hours or surgery (including arthroscopy); or a history of any form of arthritis diagnosed by a medical practitioner. A further exclusion criterion was a contraindication to MRI. Subjects who fulfilled the inclusion criteria and attended the first year of round 3 follow-up of the MCCS which commenced in 2003 were invited, and quota sampling was used whereby recruitment ceased when the target sample of approximately 300 subjects was achieved. The study was approved by The Cancer Council Victoria Human Research Ethics Committee and the Standing Committee on Ethics in Research Involving Humans of Monash University. All participants gave written informed consent.

2.1.4 Group 4 - Clinically Healthy, Pain Free Post-menopausal Women

One hundred and seventy six women, aged 40-67 were recruited from an existing cross-sectional study examining knee structure in women [173]. These women were initially recruited from a database established from the electoral roll in Victoria, Australia between April 2002 and August 2003 [311]. Women were excluded if they had OA, as defined by the American College of Rheumatology clinical criteria [1], current or past knee disease, a history of knee pain in the past five years lasting for > 24 hours; a previous knee injury requiring non-weight bearing treatment for > 24 hours or surgery (including arthroscopy); or a history of any arthritis diagnosed by a medical practitioner or contraindication to MRI. The study was approved by the Alfred Hospital Human Research Ethics Committee, and all participants gave written informed consent.

2.2 Data Collection

2.2.1 Anthropometric Data

Study participants completed a questionnaire that included information on their demographics at each time point. Weight was measured to the nearest 0.1 kg (shoes, socks, and bulky clothing removed) using a single pair of electronic scales. Height was measured to the nearest 0.1 cm (shoes and socks removed) using a stadiometer. From these data, body mass index (BMI, weight/height^2 kg m^{-2}) was calculated. These data were collected for all study populations.

2.2.2 Assessment of Knee Symptoms

These data were collected for all study population. Pain, stiffness, and function were assessed from questionnaires and by using the Western Ontario and McMaster University Osteoarthritis Index (WOMAC) [115].

2.2.3 Gait Analysis

This data was collected on 20 women within the group of healthy post-menopausal women (Group 2). Gait analyses were conducted in the gait laboratory in the Musculoskeletal Research Centre, La Trobe University, Australia. A six-camera Vicon motion analysis system (Oxford Metrics Ltd., Oxford, UK) was used to capture three-dimensional kinematic data on the dominant leg during four walking trials, at the subjects' self-selected speed to capture normal gait patterns. Ground reaction forces were measured by a Kistler 9281 force-platform (Kistler Instruments, Winterthur, Switzerland). Inverse dynamic analyses were performed using "PlugInGait" (Oxford Metrics, Oxford, UK) which is based on a previously proposed model [312], to obtain joint moments calculated about an orthogonal axis system located in the distal segment of a joint as previously described [254, 313]. Inter-ASIS (anterior superior iliac spine) distance was measured using a calliper, causing the medial-lateral and proximal-distal co-ordinates of the hip joint centre to be determined by the method previously described [312]. The ASIS to greater-trochanter measurement provided the anterior-posterior co-ordinate of the hip joint. A knee

alignment device (KAD) was used to calculate knee joint axes. The coronal plane of the thigh was defined as the plane containing the hip joint centre, knee marker and lateral KAD marker. The coronal plane of the shank contained the knee joint centre and lateral malleolus marker. The angle formed by the knee and ankle joint axes measured tibial torsion.

Foot rotation was measured about an axis perpendicular to the foot vector and the ankle flexion axis. It is defined as the angle between the foot vector and the sagittal axis of the shank, projected into the foot transverse plane. This differs from the toe-out angle, which is measured from the long axis of the foot, relative to the line of progression of the body. The foot is defined by the single vector joining the ankle joint centre to the 2nd toe. The relative alignment of this vector and the long axis of the foot is calculated from a static trial using an additional calibration marker from the heel. The foot vector is established by making two rotations about the orthogonal axis. This measure is equal to the angle between the line joining the heel marker and the toe marker, projected in the plane perpendicular to the ankle flexion axis (sagittal). The second rotation is about a foot rotation axis which is perpendicular to the foot vector and the ankle flexion axis. This measure is equal to the angle projected in the plane perpendicular to the foot rotation axis (transverse). The angle is measured between the line joining the heel and toe markers and the line joining the ankle centre and toe marker as previously described [75, 313] and according to the protocol stipulated by the Vicon technology in the gait laboratory. Positive values correspond with internal rotation (VICON Clinical Manager's User Manual). Subjects were instructed to walk barefoot at their normal pace over level ground, to capture their natural gait patterns.

2.2.4 Smoking Status

This data was collected in the population of healthy community-based men and women (Group 3) and the second population of clinically healthy, pain free postmenopausal women (Group 4). Subjects were classified as having 'ever smoked' if they reported smoking at least 7 cigarettes or seven pipes of tobacco per week for at least 1 year as previously described. If subjects did not consume this amount, they were classified as having 'never smoked'. Subjects who had 'ever smoked' were also asked about the average number of cigarettes they smoked a day and about the

number of years for which they had smoked. From this the 'pack-year' variable was calculated by averaging the number of cigarettes smoked daily, dividing by 20 (considered 1 pack) and multiplying by the number of years smoked as previously described [314].

2.2.5 Dietary Intake of fatty acids

This data was collected in the healthy community-based people group (Group 3). Dietary intake of nutrients was assessed using a food frequency questionnaire (FFQ) [315] or a 121-item FFQ developed from a study of weighed food records[316].

2.2.6 Measurement of Blood Lipids

This data was collected in the second population of clinically healthy, pain free postmenopausal women (Group 4) Each participant had a single morning fasting blood test at the time of the original study (2002-2003) approximately 1.53 years (SD 0.24 years) prior to their first knee MRI. Fasting bloods drawn at the time of recruitment were stored at -80°C until assayed. Total cholesterol was determined by the CHOD-PAP method and triglycerides (TG) by the GPO-PAP method using a Hitachi 747 analyser (Boehringer Mannheim Systems). High-Density lipoprotein (HDL) cholesterol was measured by an enzymatic colorimetric test on a Hitachi 747 analyser. The assay range is 0.1-20mg/L with intra-assay CVs of 1.34% at 0.55mg/L and 0.28% at 12.36mg/L, interassay CVs of 5.7% at 0.52mg/L and 2.5% at 10.98mg/L and a detection limit of 0.03mg/L [317]. Low-density lipoprotein (LDL) cholesterol was calculated according to a method previously described [318].

2.3 Radiography

Radiographs were obtained for the population of patients with established knee OA (Group 1) and for the first population of healthy, asymptomatic post-menopausal women (Group 2). Weight-bearing anteroposterior tibiofemoral radiograph were taken of the dominant knee (defined as the lower limb from which the subject used to step off when walking in full extension. Radiographs were independently scored by 2 trained observers (research fellows in the research group) using a published atlas to

classify disease in the tibiofemoral joint [319]. The radiographic features of tibiofemoral OA were graded in each compartment on a four-point scale (0-3) for individual features of osteophytes and JSN [319]. Intraobserver reproducibility was 0.93 for osteophytes and 0.93 for JSN. Interobserver reproducibility was 0.86 for osteophytes and 0.85 for JSN (by kappa statistic) [226]. Where both knees were symptomatic and showed changes of radiographic OA, the knee with the least severe disease was used.

Knee angles were measured from standing anteroposterior radiographs [320, 321], by a single observer (a research fellow in the research group). Lines were drawn through the middle of the femoral shaft and through the middle of the tibial shaft (Figure 2.1). The angle subtended at the point at which these two lines met in the centre of the tibial spines was based on a modification of the method of Moreland *et al.* [321] and was recently validated by Hinman et al [322] as an alternative to the mechanical axis on full-leg radiographs. The angle subtended by the lines on the medial side was measured using Osiris software (Geneva, Switzerland). Thus, an angle less than 180 degrees was more varus and an angle greater than 180 degrees more valgus. The intraobserver variability was 0.98 [254].

In addition skyline (infero-superior) views were also taken of each subject in the population of patients with established knee OA (group 1) positioned in supine with 45° of knee flexion (using a perspex positioning wedge). Femoral sulcus angles were measured from these images. The femoral sulcus angle was measured independently by two trained observers. The femoral sulcus angle was defined by lines joining the highest points of the medial and lateral condyles and the lowest point of the intercondylar sulcus (Figure 2.2) [323, 324]. The angle was measured using the software program Osiris (University of Geneva). All angles were reported in degrees. The intraclass correlation coefficient (ICC) between the two sulcus angle measurements was calculated to assess the reliability of the two sulcus angle measurements using Stata software version 9 (StataCorp 2005). The ICC was estimated to be 0.98 (95% CI 0.97 – 0.99).

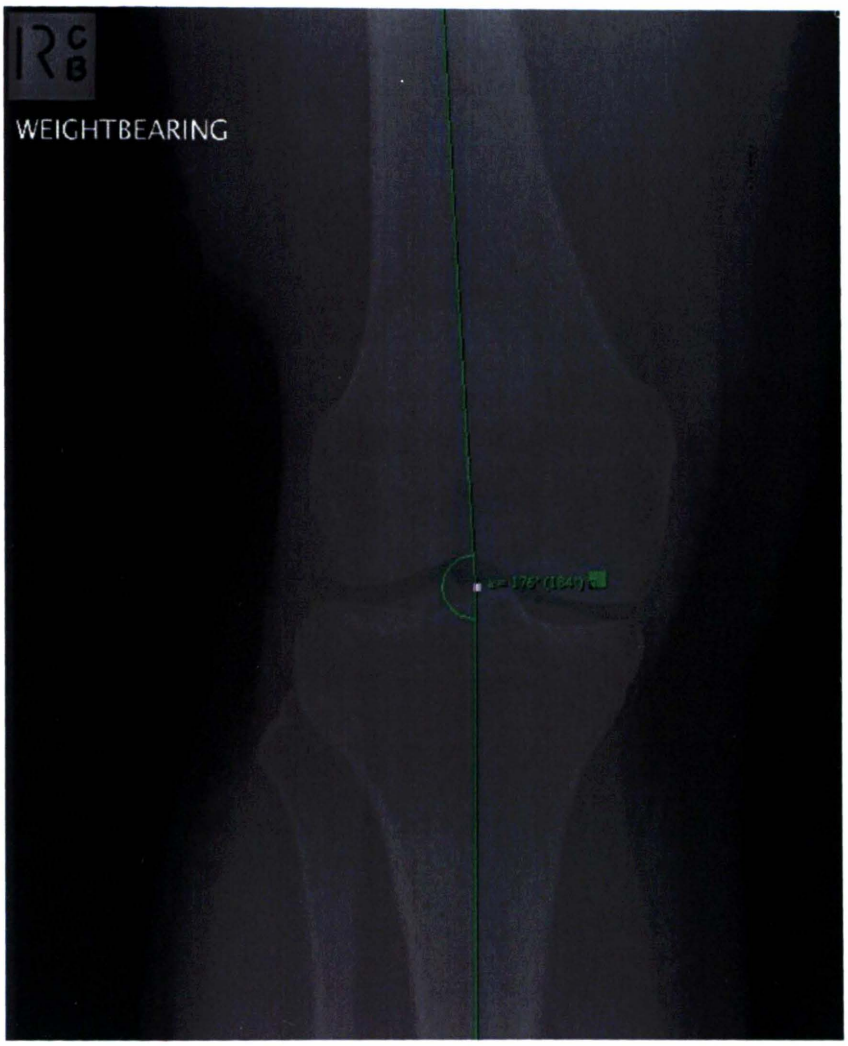


Figure 2.1. Static tibiofemoral knee angle as measured from standing AP radiographs. It is the angle subtended by the intersection of a line drawn through the middle of the femoral shaft and a line drawn through the middle of the tibial shaft [321].

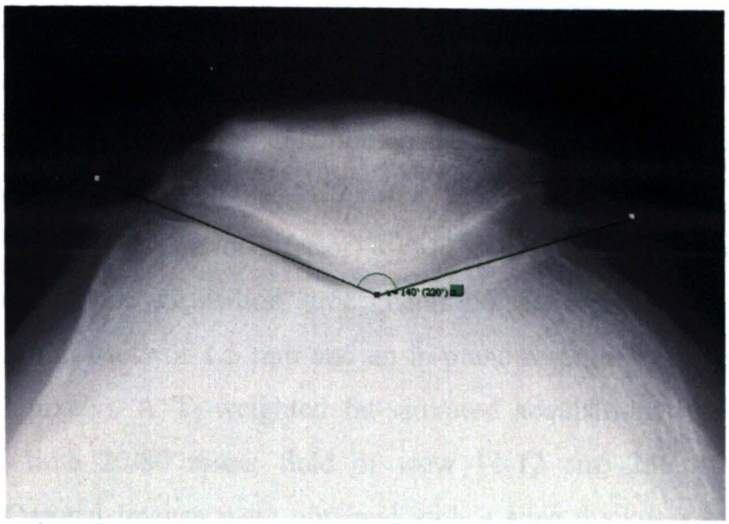


Figure 2.2 Skyline radiograph used to measure femoral sulcus angle. The femoral sulcus angle was defined by lines joining the highest points of the medial and lateral condyles and the lowest point of the intercondylar sulcus as previously described [323, 324]

2.4 Outcome Measures

Outcome measures included knee cartilage volume and its rate of change, prevalence and grade of knee cartilage defects and its change in the respective compartment, tibial plateau bone area and its rate of change and incidence and persistence or resolution of BMLs.

2.4.1 *Magnetic Resonance Imaging*

MRI was performed on the dominant knee (the healthy population groups, i.e. Group 2, 3 and 4), or the symptomatic knee (the OA group, i.e. Group 1). Knees were imaged in sagittal plane on a 1.5-Tesla whole-body magnetic resonance unit (Philips 1.5 Tesla Intera, Philips Medical Systems, Eindhoven, the Netherland, for the healthy community-based people group, i.e. Group 1; Signa Advantage HiSpeed, GE Medical Systems, Milwaukee, WI, USA, for the OA group, i.e. Group 2; Signa Advantage Echospeed, GE Medical Systems, Milwaukee, WI, USA, for the healthy men and

healthy women group, i.e. Group 3 and 4) using a commercial transmit-receive extremity coil. The following sequence and parameters were used: a T_1-weighted, fat-saturated 3D gradient recall acquisition in the steady state; flip angle 55 degrees; repetition time 58 msec; echo time 12 msec; field of view 16 cm; 60 partitions; 512 (frequency direction, superior-inferior) × 512 (phase encoding direction, anterior-posterior) matrix; one acquisition, time 11 min 56 sec. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31mm × 0.31mm (512 × 512 pixels). A T_2-weighted fat-saturated acquisition; repetition time 2200 msec; echo time 20/80 msec; field of view 11-12 cm; 256 × 128 matrix; one excitation. Coronal images were obtained with a slice thickness of 3.0 mm and an interslice gap of 0.3 mm. The image data were transferred to a workstation.

2.4.1.1 Cartilage Volume

Knee cartilage volume was determined by image processing on an independent workstation using the Osiris software (Digital Imaging Unit, University Hospital of Geneva, Geneva, Switzerland). The volumes of the individual cartilage plates (medial and lateral tibial, and patellar) were isolated from the total volume by manually drawing disarticulation contours around the cartilage boundaries on each section (Figure 2.3). These data were re-sampled by bilinear and cubic interpolation (area of 312 μm and 312 μm and 1.5 mm thickness, continuous sections) for the final 3-dimensional rendering. The volume of the particular cartilage plate was determined by summing the pertinent voxels within the resultant binary volume. The measurement was done by two independent observers within the research group with independent random cross checks blindly performed by a second trained observer (a research fellow in the research group), unpaired and blinded to subject identification, data collected, and the sequence. The CVs for cartilage volume measures were 2.1% for medial tibial, 2.2% for lateral tibial, and 2.6% for patellar cartilage [217].

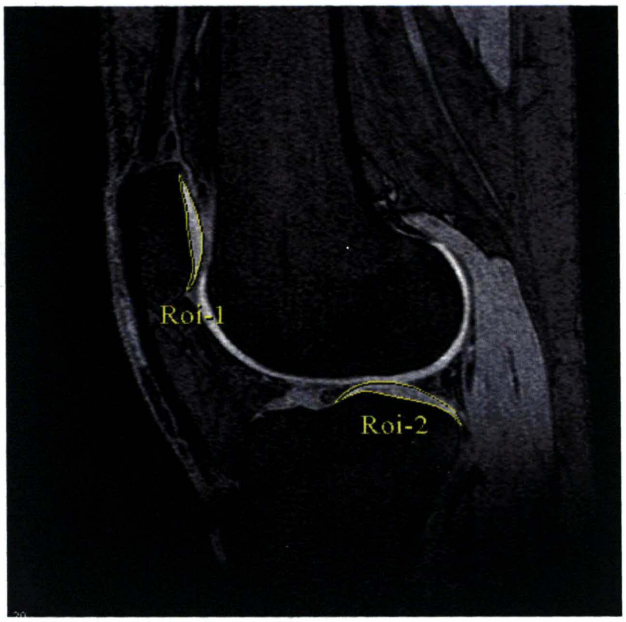


Figure 2.3 Sagittal T_1-weighted fat-saturated 3D MRI images showing measurement of cartilage volume. Patellar (Roi 1) and tibial (Roi 2) cartilage volumes were measured by manually drawing disarticulation contours around the cartilage boundaries on each section. Roi = region of interest.

2.4.1.2 Cartilage Defects

Cartilage defects were graded on MR images with a modification of a previous classification system [127, 325, 326], at medial tibial, medial femoral, lateral tibial, lateral femoral and patellar sites as follows (Figure2.4):

- Grade 0, normal cartilage;
- Grade 1, focal blistering and intracartilaginous low-signal intensity area with an intact surface and bottom;
- Grade 2, irregularities on the surface or bottom and loss of thickness of less than 50%;
- Grade 3, deep ulceration with loss of thickness of more than 50%;
- Grade 4, full-thickness cartilage wear with exposure of subchondral bone.

Cartilage surface in some images was still regular but cartilage adjacent to subchondral bone became irregular; these changes were included in the classification

system. A cartilage defect had to be present in at least two consecutive slices. The cartilage was considered to be normal if the band of intermediate signal intensity had a uniform thickness. A trained observer scored cartilage defects in duplicate (the cartilage defects were re-graded 1 month later), unpaired and blinded to subject identification, data collected, and the sequence. The defect scores at medial tibiofemoral (0-8), lateral tibiofemoral (0-8), and patellar (0-4) compartments were used as outcome measures. A prevalent cartilage defect was defined as a cartilage defect score of ≥ 2 at any site within that compartment. Intraobserver reliability (expressed as intraclass correlation coefficient, ICC) was 0.90 for the medial tibiofemoral compartment, 0.89 for the lateral tibiofemoral compartment, and 0.94 for the patellar compartment. Interobserver reliability was assessed in 50 MR images and yielded an ICC of 0.90 for the medial tibiofemoral compartment, 0.85 for the lateral tibiofemoral compartment, and 0.93 for the patellar compartment[233].

2.4.1.3 Bone Size

Medial and lateral tibial plateau bone areas were used as a measure of tibial bone size. The cross-sectional areas of medial and lateral tibial plateaus were determined by means of image processing on an independent workstation using the software program Osiris, by creating an isotropic volume from the input images, which were reformatted in the axial plane. Areas were directly measured from these axial images. Using this technique, osteophytes, if present, are not included in the area of interest. To measure the bone area of the tibial plateau, the first image that showed both tibial cartilage and subchondral bone was selected. The areas of medial and lateral tibial plateau bones were measured manually on this image and the next distal image. An average of the two areas was used as an estimate of the tibial plateau bone area (Figure 2.5). One trained observer made the measurements in duplicate, or with independent random cross checks blindly performed by a second trained observer (a research fellow in the research group), unpaired and blinded to subject identification, data collected, and the sequence. The CVs for the medial and lateral tibial plateau bone areas were 2.3% and 2.4%, respectively[217].

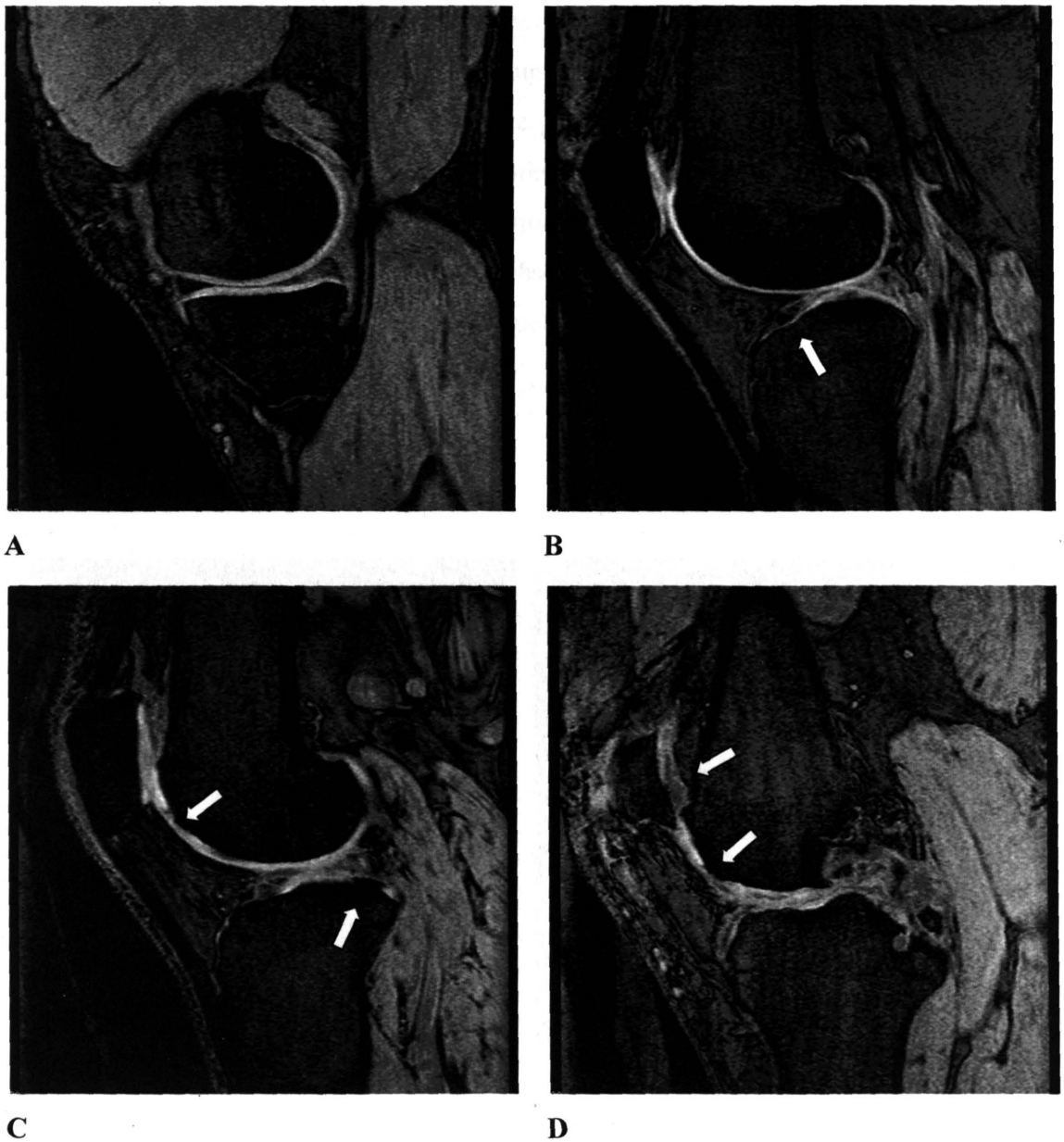
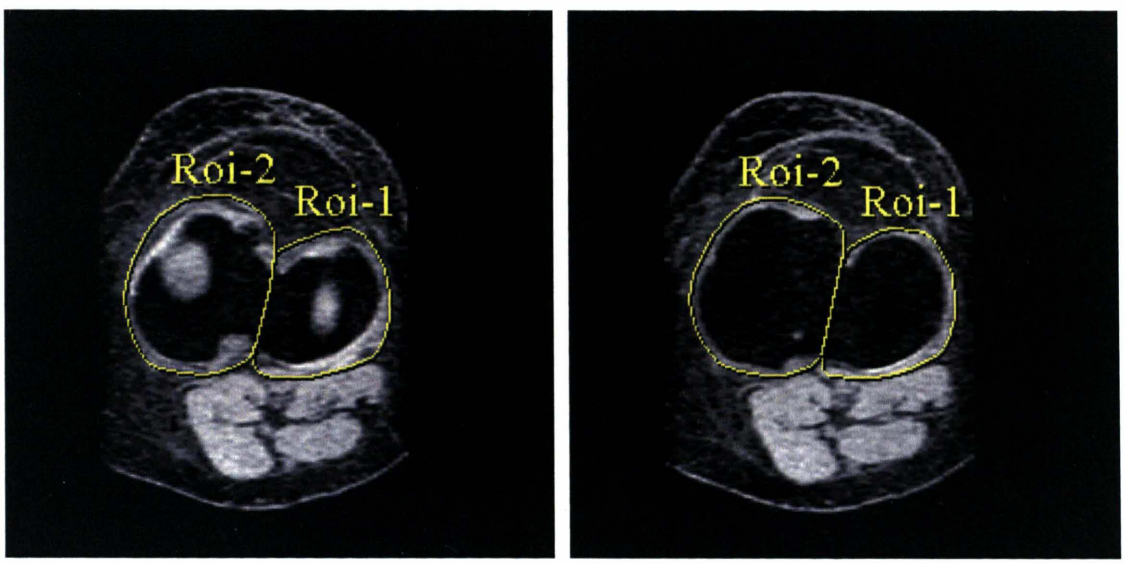


Figure 2.4 Sagittal T_1-weighted fat-saturated 3D MRI images showing the grades of cartilage defects. (A) Normal medial tibial and femoral cartilage (grade 0). (B) Normal lateral femoral and patellar cartilage (grade 0), but slight lateral tibial cartilage defect (grade 1). (C) Normal patellar cartilage (grade 0), but moderate lateral femoral cartilage defect (grade 2) and severe lateral tibial cartilage defect (grade 3). (D) Moderate medial femoral cartilage defect (grade 2) and severe patellar cartilage defect (grade 4).

Patellar bone volume was used as a measure of patellar bone size. Patellar bone volume was calculated by using the same method as for cartilage volume (Figure 2.6). Contours were drawn around the patella boundaries in images 1.5mm apart on sagittal views. Total volume was calculated for the patella due to its irregular shape, which made it difficult to identify a simpler, representative measure of patellar bone size. One trained observer made the measurements, with independent random cross checks blindly performed by a second trained observer, unpaired and blinded to subject identification, data collected, and the sequence. The CV for patellar bone volume measures was 2.2% [217].



A **B**

Figure 2.5 Axial T_1-weighted fat-saturated 3D MRI image showing measurement of tibial plateau bone area. The areas of medial (Roi 2) and lateral (Roi 1) tibial plateau bone are measured manually on the first image that shows both tibial cartilage and subchondral bone (A), and the next distal image (B). An average of the two areas is used as an estimate of the tibial plateau bone area.

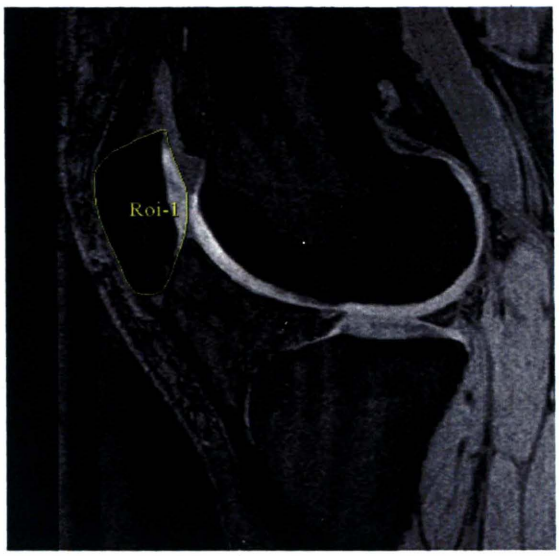
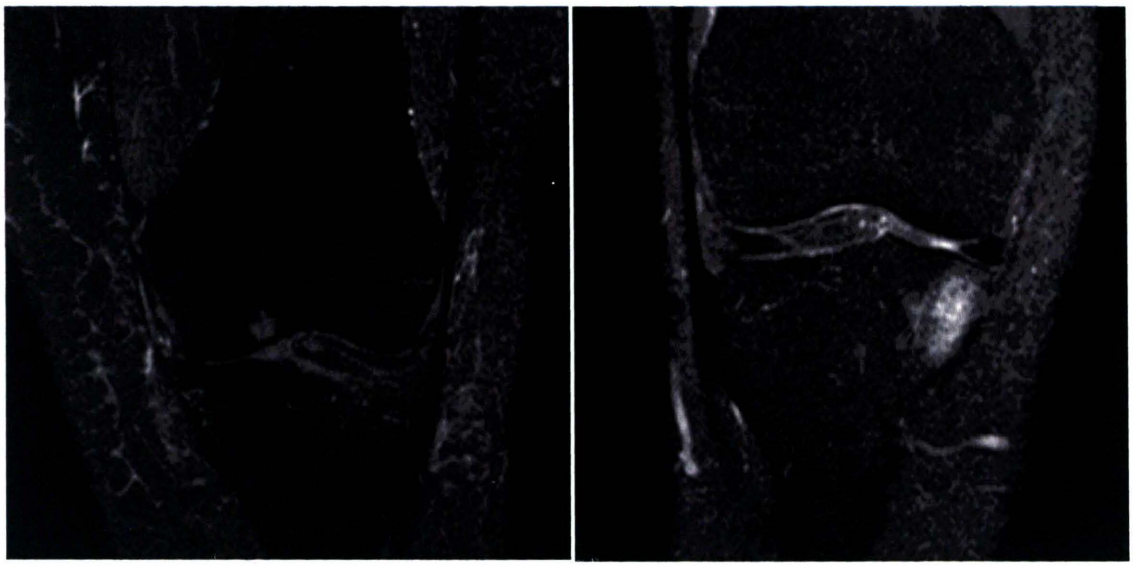


Figure 2.6 Sagittal T_1-weighted fat-saturated 3D MRI images showing measurement of patellar bone volume. Patellar bone volume was measured by manually drawing disarticulation contour around the patellar cartilage and bone boundaries (Roi 1) on each section.

2.4.1.4 Bone Marrow Lesions

BMLs were defined as areas of ill-defined areas of increased signal intensity adjacent to subcortical bone present in either the medial or lateral, distal femur or proximal tibia assessed of coronal T2-weighted fat-saturated images (Figure 2.7) [327]. Two trained observers, who were blinded to patient characteristics, as well as sequence of images, together assessed the presence of lesions for each subject. The presence or absence of a BML was determined. A lesion was defined as 'large' if it appeared on two or more adjacent slices and encompassed at least one quarter of the width of the tibial or femoral cartilage being examined from coronal images. This is comparable to the previously described 'grade 2' BML by Felson [41]. Lesions were further classified as 'very large' if they appeared on 3 or more slices. This is comparable to the previously described 'grade 3' by Felson [41] The reproducibility for determination of the BML was assessed using 60 randomly selected knee MRIs (κ value 0.88, $P < 0.001$). If a person had more than one BML underlying a cartilage plate, the BML of the highest grade was used for analysis.



A B

Figure 2.7 Coronal T_2-weighted fat-saturated MRI images showing a large (A) and a very large (B) bone marrow lesion.

2.4.1.5 Meniscal Tears

Meniscal tears were assessed in the sagittal view and confirmed in coronal and axial views by and experienced radiologists as previously described [128, 184, 185]. The presence of a tear was based on the presence of a signal, which was line shaped, brighter than the dark meniscus, and reached the surface of the meniscus at both ends within 6 defined regions (anterior horn, body and posterior horn at both medial and lateral tibiofemoral compartments). A semi-quantitative lesion assessment of meniscal tears was also performed. Our scoring system for meniscal damage referred to the accepted MRI nomenclature for meniscal anatomy, which is in accordance with arthroscopic literature [328]. The proportion of the menisci affected by tears was scored separately using the following semi-quantitative scale [185]: 0 = no damage; 1 = 1 out of 3 meniscal areas involved (anterior, middle, posterior horns); 2 = 2 out of 3 involved; 3 = all 3 areas involved. The intra- and inter-reader correlation coefficient ranged from 0.86 to 0.96 for the meniscal tears[128].

2.5 Statistical Analyses

Descriptive statistics for characteristics of each study population were tabulated. In each study, a t test was used for comparison of means. Chi-square test or Mann-Whitney U test was used to compare nominal characteristics between groups, where appropriate. Outcome measures were initially assessed for normality before being regressed against various measures of studied factors. Then linear regression was used. In terms of dichotomous outcomes, i.e. the presence/absence of tibiofemoral cartilage defects and BML, logistic regression was used. Multivariate regression models were constructed to explore the relationship between studied factors and outcome measures, adjusting for potential confounders. A P-value of less than 0.05 (two-tailed) was regarded as statistically significant. All analyses were performed using the SPSS statistical package (standard version 11.5.0 and version 14.0, SPSS, Chicago, IL, USA).

Chapter 3: The Natural History of Cartilage Defects

OA is considered to be a disease in which the whole joint is affected. However a great deal of research has been focusing on changes in articular cartilage and how they are associated with OA. These cartilaginous changes include a decrease in cartilage thickness and volume, as well as an increase in the number and size of cartilage defects.

Cartilage defects have aroused particular interest. They are found in healthy subjects as well as in those with OA [233, 234]. Their presence has been shown to predict cartilage loss [161, 163], and knee joint replacement [237] and they are significantly associated with knee symptoms [175, 239] and disease severity [237]. Given the clinical importance of cartilage defects, it is important to identify the factors associated with cartilage defects and their progression. Previous studies however, examining the natural history of cartilage defects has predominantly been performed in healthy, asymptomatic populations [163, 235, 238] and there is little data on the natural history of cartilage defects in those with OA [239, 271, 329].

The paper presented within this chapter describes the natural history of cartilage defects. It describes the progression of cartilage defects and factors associated with the progression in those with knee OA.

3.1 **Davies-Tuck ML**, Wluka AE, Wang Y, Teichtahl AJ, Jones G, Ding C, Cicuttini FM. The natural history of cartilage defects in people with knee osteoarthritis. *Osteoarthritis Cartilage* 2008; **16,** 337-42.

Over 2 years, cartilage defects tended to progress in people with symptomatic OA, with only a small percentage decreasing in severity. Increasing age and increased bone area were risk factors for progression. Interventions aimed at preventing cartilage defects from occurring and reducing their severity may result in a reduction in the severity of OA, by reducing loss of articular cartilage and subsequent requirement for knee joint replacement.

PART B: Suggested Declaration for Thesis Chapter

Monash University

Declaration for Thesis Chapter 3: The Natural History of Cartilage Defects in an Osteoarthritic Population

Declaration by candidate

In the case of Chapter 3, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Literature review, analysis and interpretation of results, manuscript draft preparation	65

The following co-authors contributed to the work. Co-authors who are students at Monash University must also indicate the extent of their contribution in percentage terms:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Anita Wluka	Study design, recruitment of subjects interpretation, draft revision	
Yuanyuan Wang	Measurement, Interpretation, draft revision	
Graeme Jones	Study Design, draft revision	
Changhai Ding	Study Design, draft revision	
Andrew Teichtahl	Draft revision	
Flavia Cicuttini	Study design, recruitment of subjects, interpretation, draft revision	

Candidate's Signature		Date

Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;

(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
(4) there are no other authors of the publication according to these criteria;
(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
(6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location(s)	Department of Epidemiology and Preventive Medicine, Monash University

[Please note that the location(s) must be institutional in nature, and should be indicated here as a department, centre or institute, with specific campus identification where relevant.]

Signature 1

Signature 2

Signature 3

Signature 4

Signature 5

Signature 6

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Osteoarthritis and Cartilage

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OSTEOARTHRITIS

The natural history of cartilage defects in people with knee osteoarthritis

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Summary

Objectives: Cartilage defects are highly prevalent in subjects with knee osteoarthritis (OA). Although they are associated with increased cartilage loss and joint replacement, there is little data on the natural history of cartilage defects. The aim of this study was to examine the progression of cartilage defects over 2 years in people with knee OA and to identify factors associated with progression.

Methods: One hundred and seventeen subjects with OA underwent magnetic resonance imaging of their dominant knee at baseline and follow-up. Cartilage defects were scored (0–4) at four sites. Bone size of the medial and lateral tibial plateau was determined. Height, weight, body mass index and physical activity were measured by standard protocols.

Results: The mean cartilage defect score increased significantly over the 2-year study period in all tibiofemoral compartments (all $P < 0.001$), except the lateral tibial compartment with age and tibial plateau bone area at baseline being predictors of progression. However, there was heterogeneity with 81% progressing at any site, 15% remaining stable and 4% decreasing.

Conclusion: Over 2 years, cartilage defects tend to progress in people with symptomatic OA, with only a small percentage decreasing in severity. Increasing age and increased bone area are risk factors for progression. Interventions aimed at preventing cartilage defects from occurring and reducing their severity may result in a reduction in the severity of OA, by reducing loss of articular cartilage and subsequent requirement for knee joint replacement.

Key words: Knee osteoarthritis, Cartilage defects, Progression, Risk factors.

Introduction

Osteoarthritis (OA) is a disease of multifactorial aetiology, which affects the entire joint. Loss of knee cartilage volume is associated with a worsening of knee symptoms[1] and knee joint replacement[2]. Defects in articular cartilage, detected using magnetic resonance imaging (MRI) are found in healthy subjects as well as in those with OA[3,4]. They have been shown to occur following trauma[5] and are associated with the radiographic criteria for OA[6–8] and correlate with symptoms[9,10]. In younger largely healthy subjects, the prevalence and severity of cartilage defects increase with age and body mass index (BMI)[11,12]. Cartilage defects are associated with decreased knee cartilage volume[3], increased rate of cartilage loss[11,13] and knee joint replacement[14].

Previous studies have focused on the natural history of cartilage defects in healthy, non-symptomatic populations[11,15,16] with little data on the natural history of cartilage defects in those with OA[9,17,18]. In a young population containing the adult offspring of people who underwent knee joint replacement for OA, articular cartilage defects progressed in 33% of people and improved in 37% over 2.3 years, however only 17% of the study population actually had radiographically diagnosed OA[15]. In contrast, in 43 subjects with chronic knee pain, of which 15 had radiographic criteria of OA, who were studied over 2 years, 66% of defects remained constant[9]. Amin *et al.* who examined a larger population, found that 60% of medial and 83% lateral cartilage remained unchanged[17,18] over at least 15 months.

Therefore, we performed a 2-year cohort study of individuals with established symptomatic knee OA to determine the natural history of cartilage defects and to identify factors that might influence this.

Materials and methods

STUDY PARTICIPANTS

Subjects with knee OA were recruited by advertising through local newspapers and the Victorian branch of the Arthritis Foundation of Australia and in collaboration with General Practitioners, Rheumatologists and Orthopaedic Surgeons[19]. The study was approved by the ethics committee of the Alfred and Caulfield hospitals in Melbourne, Australia. All patients gave informed consent. One hundred and thirty two subjects aged over 40 years who fulfilled American College of Rheumatology (ACR) clinical and radiographic criteria for knee OA[20], with pain and osteophytes present within the knee, entered the study. Subjects were excluded if any other form of arthritis was present,

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if there was any contraindication to MRI, if a total knee replacement was planned, or if they were unable to cooperate with study requirements. This population has been previously described[19].

DATA COLLECTION

At baseline, participants completed a questionnaire that included demographic data and level of current physical activity[21]. Weight was measured to the nearest 0.1 kg (shoes, socks and bulky clothing removed) using a single pair of electronic scales. Height was measured to the nearest 0.1 cm (shoes and socks removed) using a stadiometer. BMI (weight/height^2 kg/m^2) was calculated. Pain, stiffness, and function of the knee were assessed using WOMAC (Western Ontario and McMaster University Osteoarthritis Index)[22].

RADIOGRAPHIC EVALUATION

At baseline, each subject had a weight-bearing anteroposterior (AP) tibiofemoral radiograph taken of the symptomatic knee in full extension[23]. All radiographs were independently scored by two trained observers (AW and FC) using a published atlas to grade radiological features of tibiofemoral OA on a four point scale (0–3) for individual features of osteophytes and joint space narrowing where 0 = no disease and grade 3 = most severe grade as described in the atlas[24]. Intraobserver reproducibility was 0.93 for osteophytes and 0.93 for joint space narrowing. Interobserver reproducibility was 0.86 for osteophytes and 0.85 for joint space narrowing (by κ statistic)[25]. Where both knees were symptomatic and showed changes of radiographic OA, the knee with the least severe disease was used.

Measurement of static knee alignment

Knee angles were measured by a single blinded trained observer from standing AP radiographs using the software program Osiris (University of Geneva)[26]. Lines were drawn through the middle of the femoral shaft and through the middle of the tibial shaft. The angle subtended at the point at which these two lines met in the centre of the tibial spines, and was recently validated by Hinman *et al.*[27] as an alternative to the mechanical axis on full-leg radiographs. Knee angles were considered as a continuum ranging from 0° to 360°, with 0° representing extreme varus and 360° representing extreme valgus. Although these degrees of varus and valgus are not clinically observed, this range was used to avoid defining varus and valgus from an arbitrarily chosen midline value, and allow quantification of change in alignment. Intraobserver reliability [expressed as intraclass correlation coefficient (ICC)] was 0.98[28].

MRI EXAMINATION

Each subject had an MRI performed on the symptomatic knee at baseline, and were followed up by a repeated MRI performed on the same knee approximately 2 years. Knees were imaged in a sagittal plane on the same 1.5-T whole-body magnetic resonance unit (Signa Advantage HiSpeed GE Medical Systems, Milwaukee, WI, USA) using a commercial receive-only extremity coil. The following sequence and parameters were used: a T_1-weighted, fat-suppressed 3D gradient recall acquisition in the steady state; flip angle 55°; repetition time 58 ms; echo time 12 ms; field of view 16 cm; 60 partitions; 512 (frequency direction, superior–inferior) × 512 (phase encoding direction, anterior–posterior) matrix; one acquisition, time 11 min 56 s. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31 mm × 0.31 mm (512 × 512 pixels)[1,25].

Cartilage defect assessment

Cartilage defects were graded on the MR images using a validated classification system[29–31] at the medial tibial, medial femoral, lateral tibial and lateral femoral sites as previously described[3,11,14,16]. Cartilage defects were graded based on depth as follows: grade 0, normal cartilage; grade 1, focal blistering and intracartilaginous low-signal intensity area with an intact surface and bottom; grade 2, irregularities on the surface or bottom and loss of thickness of less than 50%; grade 3, deep ulceration with loss of thickness of more than 50%; grade 4, full-thickness cartilage wear with exposure of subchondral bone. A cartilage defect also had to be present in at least two consecutive slices. The baseline and follow-up cartilage defects were graded in duplicate (the cartilage defects were re-graded 1 month later), unpaired and blinded to the sequence. The defect scores at medial tibiofemoral (0–8) and lateral tibiofemoral (0–8) compartments were used in the study. A prevalent cartilage defect was defined as a cartilage defect with a score of ≥2 at any site within that compartment. A cartilage defect was defined as "progressing" if the defect score increased or "improving" if the defect score decreased over the 2-year follow-up, respectively. Intraobserver reliability (expressed as ICC) was 0.90 for the medial tibiofemoral compartment, and 0.89 for the lateral tibiofemoral compartment. Interobserver reliability was assessed in 50 MRIs and yielded an ICC of 0.90 for the medial tibiofemoral compartment, and 0.85 for the lateral tibiofemoral compartment[12,32].

Cartilage volume measurement

Tibial cartilage volume was determined by means of image processing on an independent workstation using the software program Osiris (University of Geneva) as previously described[25,33]. Two trained observers read and measured cartilage volume on each MRI, blinded to the patient's identification and study sequences as previously described[28]. The coefficient of variations (CVs) for cartilage volume measures were 3.4% for medial tibial, and 2.0% for lateral tibial cartilage[25].

Bone area measurement

Medial and lateral tibial plateau cross-sectional areas were used as a measure of bone size. These were determined using Osiris software (University of Geneva) as previously described[25,33,34]. Medial and lateral tibial plateau areas were determined by creating an isotropic volume from the input images, which were reformatted in the axial plane. Areas were directly measured from these images. The CV for bone size measures was 2.3% for medial and 2.4% for lateral tibial plateau area[25].

STATISTICAL ANALYSES

Descriptive statistics for the characteristics of the study subjects were tabulated. Paired samples *t* tests were used for comparison of means. The annual change of cartilage defect score was assessed for normality prior to linear regression techniques being used to explore the possible factors affecting annual change in tibiofemoral cartilage defect score. The severity of OA was adjusted for by including baseline cartilage volume and tibial bone plateau area within the regression model[35]. A P value of less than 0.05 (two-tailed) was regarded as statistically significant. All analyses were performed using the SPSS statistical package (standard version 14.0, SPSS, Chicago, IL, USA).

Results

One hundred and seventeen participants (89%) completed the follow-up with interpretable scans (Table I). Nine subjects were lost to follow-up: two moved overseas or interstate, three were too busy to continue in the study, two had knee surgery, one died of complications related to diabetes mellitus and chronic obstructive airways disease and one subject was too ill to continue due to multiple sclerosis. Six subjects' images were unsuitable for measurement of cartilage defects. Those lost to follow-up at 2 years were slightly taller ($P = 0.62$), weighed more ($P = 0.07$) and had slightly higher tibial cartilage volume at baseline ($P = 0.03$).

Most subjects had mild to moderate OA with 25 (20%) having severe (grade 3) medial tibiofemoral osteophytes and/or joint space narrowing and only 18 (15%) having severe lateral tibiofemoral osteophytes and/or joint space narrowing. Tibial cartilage defects were common, 56% of people had them in the medial compartment and 64% in the lateral compartment.

The change in cartilage defect score from baseline to 2-year follow-up is presented in Table II. The mean cartilage defect score increased significantly over the first 2-year study period in all tibiofemoral compartments ($P < 0.001$) except the lateral tibial compartment. The medial and lateral tibiofemoral defect scores increased in 68% and 66% of subjects; remained unchanged in 13% and 18% of subjects; and decreased in 19% and 16% of subjects, respectively. In the total tibiofemoral compartment, 81% of defect scores increased overall, 15% remained unchanged and 4% decreased ($P < 0.001$). A representative MR image of a patient showing progression of cartilage defects over 2 years is presented in Fig. 1.

Table I
Characteristics of study population at baseline

	$n = 117$
Age (years)	63.7 (10.2)
Gender (% female)	68 (58%)
Height (cm)	167.0 (8.9)
Weight (kg)	80.5 (15.0)
BMI (kg/m^2)	28.8 (5.1)
WOMAC score	
Pain	81 (44)
Stiffness	40 (22)
Function	307 (170)
Total	427 (225)
Tibiofemoral osteophytes ≥ grade 2 (%)	
Medial	24 (20%)
Lateral	34 (29%)
Joint space narrowing ≥ grade 2 (%)	
Medial	38 (32%)
Lateral	14 (12%)
Tibial plateau bone area (cm^2)	
Medial	20.8 (3.9)
Lateral	13.6 (2.6)
Tibial cartilage volume (mls)	
Medial	1.74 (0.5)
Lateral	1.92 (0.6)
Prevalence of defects ≥ grade 2 (%)	
Medial compartment	66 (56%)
Tibial	61 (52%)
Femoral	39 (33%)
Lateral compartment	64 (55%)
Tibial	64 (55%)
Femoral	19 (16%)
Cartilage defect score*	
Medial tibiofemoral	3 (1.0–8.0)
Lateral tibiofemoral	2 (1.0–7.0)
Knee angle	180.7 (5.8)

Data reported as mean [standard deviation (SD)], except where variables categorical.
*Data reported as median and range.

Factors affecting the change in the medial and lateral tibiofemoral cartilage defect score over 2 years are presented in Table III. Age was positively associated with change in medial cartilage defect score after adjusting for confounders ($P = 0.01$). Baseline cartilage defect score was negatively associated with a change in cartilage defect score for the medial and lateral compartments in univariate analysis and remained significant after adjusting for confounders ($P < 0.001$). Baseline tibial bone plateau area was positively associated with change in defects in both the medial ($P = 0.002$) and lateral compartments ($P = 0.03$) after adjusting for confounders. In the medial compartment, being female ($P = 0.01$) was associated with worsening of cartilage defects. Height, weight, and BMI were not significantly associated with the change of cartilage defects. Annual change in cartilage volume was not associated with cartilage defects over 2 years within the medial [regression coefficient 0.54 (95% confidence interval (CI) −0.23, 1.3) $P = 0.2$] or lateral [regression coefficient 0.06 (95% CI −0.7, 0.9) $P = 0.9$] compartment after adjusting for confounders. Including baseline knee alignment in the regression model did not change the results except that gender was no longer found to be significant in the medial compartment ($P \mp 0.2$). Within the medial compartment there was a trend for osteophyte grade at baseline to predict cartilage defect progression (regression coefficient 0.1 (95% CI −0.06, 0.21) P value = 0.06) after adjusting for confounders. No association between joint space narrowing and progression of cartilage defects was seen. Within the lateral compartment, osteophyte grade did not predict cartilage defect progression while a trend with joint space narrowing (regression coefficient 0.2 (95% CI −0.005, 0.36) P value = 0.06) was observed after adjusting for confounders. There was no significant association between baseline physical activity score or WOMAC score or use of non-steroidal anti-inflammatory drugs and the change in cartilage defects over 2 years (results not shown).

Discussion

In this study, we found that cartilage defects tend to progress over 2 years in people with symptomatic knee OA. Factors associated with progression of cartilage defects were increasing age and baseline tibial bone area. Although female gender was significantly associated with progression of cartilage defects, it was no longer significant after adjusting for knee alignment. Baseline cartilage defect score was negatively associated with the change in cartilage defects.

Our finding that cartilage defects generally progress over time is consistent with other published research, in both symptomatic and asymptomatic populations[9,15,16,18]. Longitudinal studies examining the natural progression of tibiofemoral cartilage defects in symptomatic populations have had contradictory findings[9,15,17,18]. Ding *et al.* showed that 33% of defects progressed and 37% improved[15]. In contrast Boegard *et al.* and Amin *et al.* showed that the majority of

Table II
Change in cartilage defect score from baseline to 2-year follow-up

	Baseline ($n = 117$)	2-Year follow-up ($n = 117$)	P value*	Increased (%)	Unchanged (%)	Decreased (%)
Medial tibial cartilage defect score	2.0 (1.1)	2.3 (1.4)	**<0.001**	32	67	1
Medial femoral cartilage defect score	1.4 (1.3)	2.0 (0.9)	**<0.001**	62	17	21
Medial tibiofemoral cartilage defect score	3.4 (2.3)	4.4 (1.6)	**<0.001**	68	13	19
Lateral tibial cartilage defect score	1.8 (0.8)	1.9 (1.1)	0.30	33	41	26
Lateral femoral cartilage defect score	0.8 (0.9)	1.5 (0.9)	**<0.001**	62	37	1
Lateral tibiofemoral cartilage defect score	2.5 (1.7)	3.4 (1.3)	**<0.001**	66	18	16
Total cartilage defect score	5.9 (3.0)	7.7 (2.7)	**<0.001**	81	15	4

Cartilage defect score at 2-year follow-up available for 116 subjects.
*Comparison between baseline and 2-year follow-up cartilage defect score, determined by paired t test.

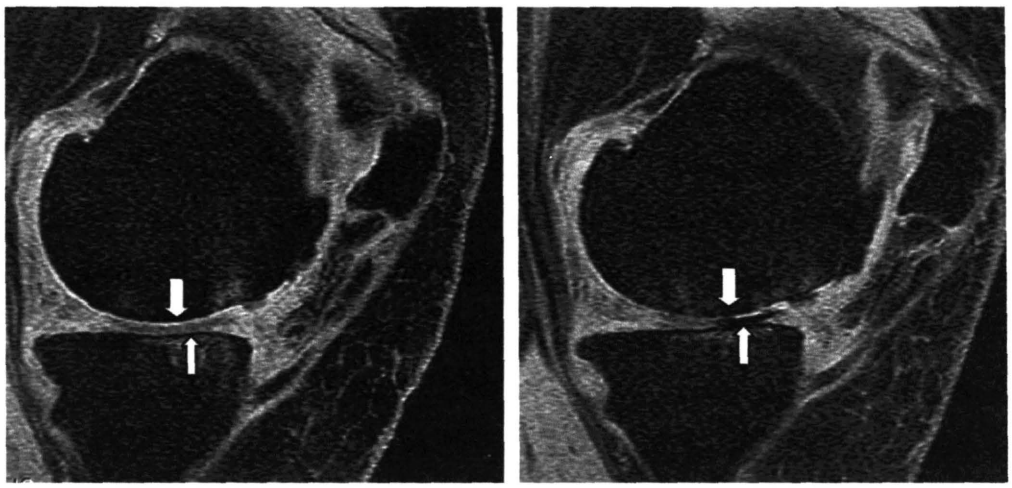

Fig. 1. MR image showing a cartilage defect score at both medial tibia and medial femur progressed from grade 3 at baseline to grade 4 at follow-up.

defects remain unchanged over time[9,18]. However, within these studies either not all participants had radiographically diagnosed OA[9,15] or they used only semi-quantitative methods and were unable to determine the relationship between cartilage volume, joint size and change in defects[17,18].

Our findings are also consistent with other studies that suggest cartilage repair occurs, as shown by regression of cartilage defects[15,16], especially amongst the most severe lesions[16]. Although these findings may suggest cartilage repair, it is also possible that the observation of improvement of cartilage defects may be in part, due to measurement error. The reproducibility of our scoring of cartilage defects was high and for a cartilage defect to be classified as present, it had to be observed in at least two consecutive MRI images, where the slice thickness was 1.5 mm. Thus partial volume averaging and errors in our grading are likely to only, in part, explain the observed improvement in defects and equally result in an apparent increase in cartilage defects. This finding of a negative association between baseline cartilage defect score and cartilage defect progression may also reflect a regression to the mean or ceiling effect. However, we have previously shown that cartilage defects progress more rapidly in the early stages and are less likely to progress when they are more severe[16]. Since these results replicate those found in a different population, this makes them more biologically plausible[15]. These findings, taken together with experimental studies in animals showing that cartilage had significant capacity for self-repair of small sized defects (3–6 mm in diameter) with hyaline or fibrocartilage[36,37] indicate the possibility that this finding reflects the reversible nature of cartilage defects in OA progression.

We have previously shown that age and gender are associated with cartilage defects in cross-sectional studies of people with OA[12,14,32] and the progression of defects[15,16] in a healthy population. In this study, we found cartilage defects were more likely to progress in women, compared to men.

Table III
Factors affecting annual change in cartilage defect scores

	Univariate analyses, regression coefficient* (95% CI)	*P* value	Multivariate analyses, regression coefficient† (95% CI)	*P* value
Medial tibiofemoral cartilage defects				
Age (years)	0.006 (−0.01, 0.02)	0.45	0.01 (0.003, 0.024)	**0.01**
Gender‡	0.25 (−0.06, 0.56)	0.12	0.41 (0.09, 0.73)	**0.01**
BMI (kg/m^2)	0.008 (−0.02, 0.04)	0.60	0.005 (−0.02, 0.03)	0.61
Baseline cartilage defect score	−0.27 (−0.32, −0.23)	**<0.001**	−0.34 (−0.41, −0.27)	**<0.001**
Baseline tibial cartilage volume (ml s)	0.296 (−0.03, 0.62)	0.07	−0.06 (−0.35, 0.23)	0.68
Baseline tibial plateau bone area (cm^2)	−0.06 (−0.09, -0.02)	**0.001**	0.07 (0.03, 0.12)	**0.002**
Lateral tibiofemoral cartilage defects				
Age (years)	0.001 (−0.013, 0.015)	0.90	0.009 (−0.002, 0.02)	0.13
Gender‡	−0.39 (−0.68, −0.11)	**0.01**	0.002 (−0.33, 0.33)	0.9
BMI (kg/m^2)	−0.001 (−0.03, 0.03)	0.92	0.004 (−0.02, 0.03)	0.73
Baseline cartilage defect score	−0.314 (−0.379, −0.249)	**<0.001**	−0.35 (−0.44, −0.26)	**<0.001**
Baseline tibial cartilage volume (ml s)	0.574 (0.343, 0.805)	**<0.001**	0.05 (−0.20 0.31)	0.69
Baseline tibial plateau bone area (cm^2)	−0.01 (−0.06, 0.03)	0.59	0.06 (0.004, 0.11)	**0.03**

*Change in cartilage defect score per unit increase in respective variable.

†Change in cartilage defects score per unit increase in respective variable after adjusting for age, gender, BMI, baseline cartilage defect score, baseline cartilage volume and baseline tibial plateau area in regression equation.

‡Males = 0 and females = 1.

The female sex has been well established as a risk factor for OA[38,39] and our results are consistent with this. However when knee alignment, a risk factor for cartilage defects[40], was included in the model, the association of gender was no longer significant suggesting that the effect of gender on cartilage defects may be mediated *via* knee alignment.

In this study, we found that baseline tibial bone plateau area was positively associated with a change in cartilage defects over 2 years. Bone size has been previously identified as a risk factor for both the prevalence and severity of cartilage defects in healthy[3,14] and OA populations[41] and more recently for the progression of cartilage defects in healthy individuals[15]. Increased bone size or expansion may result in splitting of articular cartilage and the progression of cartilage defects[3,15,42]. Tibial subchondral bone area therefore predicts cartilage defects which, in turn, predict loss of cartilage volume[13]. As cartilage loss is the hallmark of established OA, with 60% of cartilage lost by end-stage knee OA[2], tibial subchondral bone area expansion may be a primary event in OA. Consistent with this, knee cartilage defect severity was positively associated with urinary levels of C-terminal crosslinking telopeptide of type II collagen (CTX-II), a specific index for cartilage breakdown[3]. These results, together with the results of the current study suggest that prevention of tibial subchondral bone expansion and cartilage defects at an early stage may prevent the development of established knee OA[13,15].

This study has a few potential limitations. Although these include loss to follow-up which introduces bias, there were no significant differences between those who completed the study and those who did not in terms of previously identified risk factors for OA (age and BMI)[12,32]. This study also examined people with symptomatic OA, and therefore these results may not be generalisable to the asymptomatic population. Furthermore, although the grading scale we used is highly reproducible, and correlated with histological[3] and arthroscopic finding[29,31], it is based only on defect depth. Other scales include defect diameter[43] and a new 8-point grading scale [Whole-Organ MRI Score (WORMS)] which assesses cartilage defects by depth and width, in each of 15 articular surface regions[44]. This expanding scale system may be more capable of capturing different patterns of regional cartilage loss but is not strictly linear as focal defects progress to diffuse cartilage thinning thus the scale may be more diffuse than our scale which focuses purely on defect depth[45]. Finally, we were unable to examine the relationship between bone marrow lesions and meniscal damage on cartilage defect progression in this study as these could not be measured from the sequences examined in this study.

In conclusion, cartilage defects tend to progress in people with symptomatic OA with only a small percentage decreasing. Increasing age, female gender and increased bone area are risk factors for progression of cartilage defects. As cartilage defects are associated with cartilage loss and joint replacement, interventions aimed at reducing tibial bone size may have a role in reducing progression of cartilage defects and warrant further investigation.

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Chapter 4: The Significance of Meniscal Tears

Meniscal tears have also been identified as an important risk factor for the development and progression of knee OA [179, 185]. Among people with OA, tears in the menisci are associated with loss of cartilage and progression of symptomatic knee OA [83, 128, 179, 182, 185, 330]. While meniscal tears have also been identified in symptomatic and/or early OA individuals [184], the prevalence and significance of meniscal tears in these populations remains to be studied. While predominantly thought to be present due to injury, meniscal tears have also been identified in the absence of knee trauma [85]. Due to the clinical significance of meniscal tears, determining which modifiable factors are associated with meniscal lesions, even among people with no clinical knee OA, may help to better understand the pathogenesis of knee OA and develop preventative strategies. Recently, there has been increasing interest in the role of gait parameters in studies examining knee joint morphology and the genesis of knee OA and pain [75, 252, 254] and as they are the major determinant of loading passing through the knee joint they may represent one such modifiable factor.

The two papers presented within this chapter therefore describe the prevalence and significance of meniscal tears and also the relationship between gait parameters and meniscal tears in a cohort of asymptomatic women.

The first paper reports the prevalence of meniscal tears in a healthy pain free population of post-menopausal women and investigates whether meniscal tears in this population are associated with changes in cartilage volume and defects and tibial plateau bone area over two years. We report that meniscal tears are common in asymptomatic post-menopausal women, increase with age and are associated with greater tibial plateau bone area.

4.1 **Davies-Tuck ML**, Martel-Pelletier J, Wluka AE, Pelletier J-P, Ding C, Jones G, Davis SR and Cicuttini FM. Meniscal Tear and Increased Tibial Plateau Bone Area in Healthy Post-Menopausal Women. *Osteoarthritis Cartilage* **2008; 16,** 268-71.

The second paper examines whether modifiable gait parameters are associated with the presence and severity of meniscal lesions among women with no clinical knee OA.

In this cross-sectional study, we have demonstrated that medial meniscal tears are associated with changes in biomechanical factors acting on the medial tibiofemoral compartment during level walking. These data may suggest that gait parameters are associated with meniscal damage, although longitudinal studies will be required to clarify whether gait abnormalities predate meniscal lesions, or vice versa and therefore whether modification of gait patterns may be helpful.

4.2 **Davies-Tuck ML**, Wluka AE, Teichtahl AJ, Martel-Pelletier J, Pelletier J-P, Jones G, Ding C, Davis SR and Cicuttini FM. Association between Meniscal Tears and the Peak External Knee Adduction Moment and Foot Rotation During Level Walking in Postmenopausal Women without Knee Osteoarthritis: A Cross sectional Study **Arthritis Research & Therapy 2008; 10,** R58.

PART B: Suggested Declaration for Thesis Chapter

Monash University

Declaration for Thesis Chapter 4: Meniscal Tear and Increased Tibial Plateau Bone Area in Healthy Post-Menopausal Women

Declaration by candidate

In the case of Chapter 4, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Literature review, analysis and interpretation of results, manuscript draft preparation	65

The following co-authors contributed to the work. Co-authors who are students at Monash University must also indicate the extent of their contribution in percentage terms:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Martel-Pelletier J	Data acquisition, draft revision	
Anita Wluka	Study design, recruitment of subjects interpretation, data collection, draft revision	
Pelletier JP	Data acquisition, draft revision	
Changhai Ding	Draft revision	
Graeme Jones	Draft revision	
Davis S	Study design, recruitment of subjects, data collection, draft revision	
Flavia Cicuttini	Study design, recruitment of subjects, data collection, interpretation, draft revision	

Candidate's Signature		Date

Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.

(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
(4) there are no other authors of the publication according to these criteria;
(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
(6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location(s) Department of Epidemiology and Preventive Medicine, Monash University

[Please note that the location(s) must be institutional in nature, and should be indicated here as a department, centre or institute, with specific campus identification where relevant.]

Signature 1

Signature 2

Signature 3

Signature 4

Signature 5

Signature 6

Signature 7

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Osteoarthritis and Cartilage | ICRS International Cartilage Repair Society | OA OSTEOARTHRITIS

Brief report

Meniscal tear and increased tibial plateau bone area in healthy post-menopausal women[1]

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Summary

Objective: Meniscal tears detected using magnetic resonance imaging (MRI) have been identified as a risk factor for the development and progression of Osteoarthritis, however the prevalence and significance of meniscal tears in healthy, asymptomatic adults remains to be studied. We investigated the prevalence of meniscal tears in a healthy pain free population of post-menopausal women and whether meniscal tears in this population are associated with changes in cartilage volume and defects and tibial plateau bone area over 2 years.

Methods: Fifty-seven post-menopausal women underwent MRI of their dominant knee at baseline line and approximately 2 years later to assess meniscal tears, cartilage volume, cartilage defects and tibial plateau bone area.

Results: Forty-six percent of women had a meniscal tear in either the medial and/or lateral compartment. Women who had a tear were older ($P = 0.01$) and had more lateral cartilage defects ($P = 0.02$). Medial meniscal tear was associated with 103 mm^2 greater tibial plateau bone area within the medial [95% confidence of interval (CI) 6.2, 200.3; $P = 0.04$] and a lateral meniscal tear with a 120 mm^2 greater area within the lateral compartment (95% CI 45.5, 195.2; $P = 0.002$).

Conclusion: This study demonstrates that meniscal tears are common in asymptomatic post-menopausal women and that they become more common with age. Meniscal tears were also associated with greater tibial plateau bone area but not cartilage volume, providing support to the hypothesis that tibial plateau bone changes occur before significant pathological changes in cartilage. Whether increased tibial plateau bone area predisposes to an increased risk of degenerative meniscal tears or whether it is a consequence of altered biomechanical forces in relation to meniscal tear will need to be determined.

Key words: Meniscal tears, Healthy post-menopausal women, Cartilage volume/defect, Bone area.

Osteoarthritis (OA) is a disease of multifactorial aetiology which affects the entire joint. Meniscal tears detected using magnetic resonance imaging (MRI) have been identified as a risk factor for the development and progression of OA[1,2]. Previous studies examining meniscal tears have mainly been performed on osteoarthritic populations or in populations that have undergone meniscectomy[1–7]. These studies have linked tears in the meniscus to loss of articular cartilage and progression of symptomatic knee OA[1–3,6,8]. However, there has been only one study examining the relationship between meniscal tear and knee structures in healthy individuals[9]. In this cross-sectional study, which consisted of a predominantly non-osteoarthritic population, meniscal tear prevalence was found to be associated with higher cartilage defect scores, less tibial cartilage volume and increased tibial bone area[9]. However, in this study,

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meniscal tears were mainly present in those with knee pain and/or radiographic OA. Thus, the prevalence and significance of meniscal tears in healthy, asymptomatic adults remain to be studied. In the present study, we therefore investigated the prevalence of meniscal tears in a healthy pain free population of post-menopausal women and whether meniscal tears in this population are associated with changes in cartilage volume and defects and tibial plateau bone area over 2 years. We report that meniscal tears are common in asymptomatic post-menopausal women, increase with age and are associated with greater tibial plateau bone area.

Methods

SUBJECTS

The population for this study has been previously described[10]. In brief, post-menopausal women aged over 50 years were recruited through private consulting clinics and advertising in the media. Exclusion criteria were inflammatory arthritis, previous knee joint replacement, malignancy, fracture in the last 10 years, and any contraindication to MRI.

DATA COLLECTION

At baseline, participants completed a questionnaire to obtain demographic information. Weight was measured to the nearest 0.1 kg (shoes, socks and bulky clothing removed) using a single pair of electronic scales. Height was measured to the nearest 0.1 cm (shoes and socks removed) using a stadiometer. Body mass index (BMI) (weight/height^2, kg/m^2) was calculated. Pain, stiffness, and function of the knee were assessed using WOMAC (Western Ontario and McMaster University OA Index)[11].

MRI AND THE MEASUREMENT OF CARTILAGE VOLUME AND CARTILAGE DEFECTS

MRI was performed on the dominant knee as previously described[12] at baseline and 2 years later. The following sequence and parameters were used: a T1-weighted fat suppressed three-dimensional (3D) gradient recall acquisition in the steady state; flip angle 55°; repetition time 58 ms; echo time 12 ms; field of view 16 cm; 60 partitions; 512 (frequency direction, superior–inferior) × 512 (phase encoding direction, anterior–posterior) matrix; one acquisition, time 11 min 56 s. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31 mm × 0.31 mm (512 × 512 pixels).

Medial and lateral tibial plateau cartilage volumes were determined by image processing on an independent workstation using the Osiris software (Geneva, Switzerland) as reported previously[12,13] at baseline and 2 years later. The cartilage volumes of the tibial plateaus were performed by manually drawing disarticulation contours around the cartilage boundaries. The coefficient of variations (CVs) for the medial and lateral tibial cartilage volumes were 3.4% and 2.0%, respectively[13]. Medial and lateral tibial bone areas were determined by creating an isotropic volume from the input images that were reformatted in the axial plane and directly measured from these images at baseline and 2 years later. Areas were directly measured from these images by manually outlining the bone surface excluding osteophytes. CVs for medial and lateral tibial bone areas were 2.3% and 2.4%, respectively[13]. Tibiofemoral cartilage defects were graded on the MR images with a classification system that has been previously described[14] at baseline and 2 years later. Cartilage defects were graded based on depth as follows: grade 0, normal cartilage; grade 1, focal blistering and intra-cartilaginous low-signal intensity area with an intact surface and bottom; grade 2, irregularities on the surface or bottom and loss of thickness of less than 50%; grade 3, deep ulceration with loss of thickness of more than 50%; grade 4, full-thickness cartilage wear with exposure of subchondral bone. Intra-observer reliability (expressed as intra-class correlation coefficient [ICC]) was 0.85[14].

MENISCAL TEAR ASSESSMENT

Meniscal tears were assessed in the sagittal view and confirmed in coronal and axial views as previously described[2,5,9] at baseline. The presence of a tear was based on the presence of a signal, which was line shaped, brighter than the dark meniscus, and reached the surface of the meniscus at both ends. The intra- and inter-reader correlation coefficient ranged from 0.86 to 0.96 for the meniscal tears[5].

STATISTICAL ANALYSES

Descriptive statistics for the characteristics of the study subjects were tabulated. Paired samples *t* tests were used for the comparison of means. Chi-squared analysis was used to compare the prevalence of cartilage defects between those with and without meniscal tears. The annual change of cartilage defect score was assessed for normality prior to linear regression techniques being used to explore the possible factors affecting annual change in tibiofemoral cartilage defect score. The severity of OA was adjusted for by including baseline cartilage volume and tibial bone plateau area within the regression model[15]. A *P* value of less than 0.05 (two-tailed) was regarded as statistically significant. All analyses were performed using the SPSS statistical package (standard version 14.0, SPSS, Chicago, IL, USA).

Results and discussion

Eighty-one participants entered the study. Meniscal tear measurements were performed for 74 participants at baseline and 57 participants completed the follow-up at 2 years. The characteristics of the baseline population are provided in Table I. There were no significant differences in age, BMI, baseline cartilage volume and defects, tibial plateau area and meniscal tears between those who completed the study and those lost to follow-up (data not shown).

Forty-six percent of post-menopausal women had a meniscal tear in either the medial and/or lateral compartments, with 36% having a medial and 22% having a lateral meniscal tear. Nine women had a tear in both the medial and lateral compartments. Those with meniscal tears were older, but had similar BMIs, had significantly higher lateral tibiofemoral defects ($P = 0.02$), lateral tibiofemoral defect scores ($P = 0.03$) and tibial plateau bone area at both medial and lateral compartments (Table I). Interestingly, after adjusting for confounders only the relationship between the presence of meniscal tear and baseline tibial plateau bone area persisted (Table II). Medial meniscal

Table I
Characteristics of subjects with and without baseline meniscal tear

	Meniscal tear present ($n = 34$)	No meniscal tear ($n = 40$)	*P* value
Age (years)	58.8 (6.0)	55.5 (4.3)	**0.01**
Height (m)	1.65 (0.07)	1.63 (0.07)	0.3
Weight (kg)	71 (13.6)	69 (12)	0.51
BMI (kg/m^2)	26.1 (5.0)	26 (4.5)	0.9
Prevalence of meniscal tear*			
Medial	27 (36%)		
Lateral	16 (22%)		
Cartilage volume (μl)			
Medial	1580 (328)	1493 (274)	0.2
Lateral	2004 (456)	2068 (337)	0.47
Tibiofemoral cartilage defects prevalence*			
Medial	27 (36%)	29 (39%)	0.5‡
Lateral	22 (30%)	15 (20%)	**0.02**‡
Tibiofemoral cartilage defect score†			
Medial	2 (1–6)	2 (1–4)	0.33
Lateral	2 (1–8)	1 (0–3)	**0.03**
Tibial bone area (mm^2)			
Medial	1790 (212)	1672 (180)	**0.01**
Lateral	1146 (122)	1053 (129)	**0.002**

Mean and SD unless noted. *P* value for two-tailed *t* test except where noted.

*Number (%).

†Median and range.

‡Chi-squared.

tear was associated with 103 mm^2 greater tibial plateau bone area within the medial ($P = 0.04$) and a lateral meniscal tear with a 120 mm^2 greater area within the lateral compartment ($P = 0.002$). A trend towards a negative association in the lateral compartment between meniscal tear and cartilage volume was also found ($P = 0.07$) (Table II). The cross-sectional relationship between meniscal tears and osteophytes was also examined, however, no significant association was seen (data not shown).

There was no significant association between the presence of a meniscal tear and change in tibial cartilage volume, tibial plateau bone area and tibiofemoral defects over 2 years (Table II). However, in the medial compartment there was a trend towards the presence of medial meniscal tear at baseline and an increase in medial tibiofemoral defect score over 2 years ($P = 0.08$).

This study demonstrates that meniscal tears are common in asymptomatic post-menopausal women and that they become more common with age. Meniscal tears were also associated with greater tibial plateau bone area but not osteophyte severity, and trends between prevalence of meniscal tear at baseline and decreased lateral cartilage volume as well as with the progression of the tibiofemoral cartilage defects over 2 years were also found.

Our finding that meniscal tears are present in asymptomatic individuals and become more common with increasing age is consistent with a previous study conducted in a largely non-osteoarthritic population[9]. However, our prevalence of 46% is lower than that reported for the previous cohort, where 72% of individuals had tear at any site. On the other hand, when medial and lateral compartments are looked at separately, data are similar in that, in our study, a prevalence of 36% is found in the medial and 22% in the lateral compartment compared to 19–40% and 21–44% having meniscal tears in the medial and lateral compartments, respectively[9]. The higher prevalence at any site could be explained by the fact that in the previous study[9] about half of the individuals were the adult offspring of individuals who had undergone a knee replacement for primary knee OA. Furthermore 61% of those with meniscal tears had knee pain and 23% radiographic OA[9]. Moreover, in the present study, meniscal tears were found to be associated with greater tibial plateau bone area and a trend towards an increase in cartilage defects over the 2 years was observed, which is consistent with the study by Ding *et al.*[9]. In contrast, our study also showed a negative association with lateral cartilage volume. It may be that a meniscal tear leads to altered walking gait and possibly reduced loading on cartilage which is required for cartilage health. Alternatively, it may be that reduced cartilage volume leads to greater forces on the meniscus during walking leading to a tear. However, longitudinal studies examining incident tears and changes in cartilage volume would be required to assess which change happens first.

This study thus provides support to the hypothesis that tibial plateau bone changes occur before significant pathological changes in cartilage. The question that remains is whether increased tibial plateau bone area predisposes to an increased risk of degenerative meniscal tears or whether

Table II
Associations between meniscal tear at baseline and change over 2 years

	Univariate analysis	*P* value	Multivariate analysis	*P* value
At baseline				
Cartilage volume (μl)				
Medial	100.5 (−43.6, 244.6)	0.17	62 (−75.5, 199)*	0.4
Lateral	−151 (−362, 61.2)	0.16	−204 (−422, 147)*	0.07
Tibiofemoral defects (yes/no)				
Medial	2.44 (0.7, 8.4)	0.16	2.9 (0.8, 10.6)†	0.11
Lateral	2.71 (0.83, 8.8)	0.097	2.4 (0.7, 8.3)†	0.2
Tibial bone area (mm^2)‡				
Medial	106 (11.2, 201.3)	**0.03**	103 (6.2, 200.3)‡	**0.04**
Lateral	115.8 (45, 186)	**0.002**	120 (45.5, 195.2)‡	**0.002**
Annual change over 2 years				
Cartilage volume (μl)				
Medial	4.65 (−27, 36.3)	0.77	−6.1(−35.8, 23.6)§	0.7
Lateral	−20.9 (−75.5, 33.5)	0.4	−17.1 (−71.9, 37.7)§	0.5
Tibiofemoral defect score				
Medial	0.04 (−0.2, 0.25)	0.7	0.15 (−0.02, 0.32)‖	0.08
Lateral	−0.2 (−0.6, 0.2)	0.4	−0.02 (−0.2, 0.2)‖	0.9
Tibial bone area (mm^2)				
Medial	−16.9 (−35.7, 1.7)	0.07	−10.6 (−29.1, 7.8)¶	0.25
Lateral	8.6 (−16, 33.2)	0.5	18.7 (−7.7, 45.2)¶	0.2

*Difference in cartilage volume (μl) if meniscal tear is present compared to where one is absent after adjusting for age, BMI, and bone area.

†Odds ratio for tibiofemoral defects being present where a meniscal tear is present after adjusting for age, BMI and baseline cartilage volume.

‡Difference in tibial bone area (mm^2) if meniscal tear is present compared to where one is absent after adjusting for age and BMI.

§Annual change in tibial cartilage volume (μl) if a meniscal tear is present compared to where one is absent after adjusting for age, BMI and baseline cartilage volume.

‖Annual change in tibiofemoral defect score if a meniscal tear is present compared to where one is absent at baseline after adjusting for age, BMI, baseline cartilage volume and baseline cartilage defect score.

¶Annual change in tibial bone area (mm^2) if meniscal tear is present compared to where one is absent at baseline after adjusting for age, BMI and baseline tibial bone area.

it is a consequence of altered biomechanical forces in relation to meniscal tear.

Conflict of interest

None.

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PART B: Suggested Declaration for Thesis Chapter

Monash University

Declaration for Thesis Chapter 4: Association between Meniscal Tears and the Peak External Knee Adduction Moment and Foot Rotation During Level Walking in Postmenopausal Women without Knee Osteoarthritis: A Cross sectional Study

Declaration by candidate

In the case of Chapter 4, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Literature review, analysis of data, interpretation of results and manuscript draft preparation	65

The following co-authors contributed to the work. Co-authors who are students at Monash University must also indicate the extent of their contribution in percentage terms:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Anita Wluka	Study design, recruitment of subjects interpretation, data collection, draft revision	
Andrew Teichtahl	Measurement of gait variables, interpretation and draft revision	
Martel-Pelletier J	Data acquisition, draft revision	
Pelletier JP	Data acquisition, draft revision	
Graeme Jones	Draft revision	
Changhai Ding	Draft revision	
Davis S	Study design, data collection, draft revision	
Flavia Cicuttini	Study design, recruitment of subjects, data collection, interpretation, draft revision	

Candidate's Signature		Date

Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
(4) there are no other authors of the publication according to these criteria;
(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
(6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location(s) Department of Epidemiology and Preventive Medicine, Monash University

[Please note that the location(s) must be institutional in nature, and should be indicated here as a department, centre or institute, with specific campus identification where relevant.]

Signature 1
Signature 2
Signature 3
Signature 4
Signature 5
Signature 6
Signature 7
Signature 8

Research article

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Association between meniscal tears and the peak external knee adduction moment and foot rotation during level walking in postmenopausal women without knee osteoarthritis: a cross-sectional study

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Abstract

Introduction Meniscal injury is a risk factor for the development and progression of knee osteoarthritis, yet little is known about risk factors for meniscal pathology. Joint loading mediated via gait parameters may be associated with meniscal tears, and determining whether such an association exists was the aim of this study.

Methods Three-dimensional Vicon gait analyses were performed on the dominant knee of 20 non-osteoarthritic women, and the peak external knee adduction moment during early and late stance was determined. The degree of foot rotation was also examined when the knee adductor moment peaked during early and late stance. Magnetic resonance imaging was used to determine the presence and severity of meniscal lesions in the dominant knee.

Results The presence ($P = 0.04$) and severity ($P = 0.01$) of medial meniscal tears were positively associated with the peak external knee adduction moment during early stance while a trend for late stance was observed ($P = 0.07$). They were also associated with increasing degrees of internal foot rotation during late stance, independent of the magnitude of the peak external knee adduction moment occurring at that time ($P = 0.03$). During level walking among healthy women, the presence and severity of medial meniscal tears were positively associated with the peak external knee adduction moment. Moreover, the magnitude of internal foot rotation was associated with the presence and severity of medial meniscal lesions, independent of the peak knee adductor moment during late stance.

Conclusion These data may suggest that gait parameters may be associated with meniscal damage, although longitudinal studies will be required to clarify whether gait abnormalities predate meniscal lesions, or *vice versa*, and therefore whether modification of gait patterns may be helpful.

Introduction

Meniscal injury is recognised as a significant risk factor for the development and progression of knee osteoarthritis (OA) [1,2] and may be present with or without a history of significant trauma when assessed via magnetic resonance imaging (MRI) [2-6]. In subjects without clinical knee OA, meniscal tears have been associated with structural changes associated with OA, including the presence of more severe cartilage defects,

ASIS = anterior superior iliac spine; BMI = body mass index; CI = confidence interval; KAD = knee alignment device; KL = Kellgren-Lawrence; MRI = magnetic resonance imaging; OA = osteoarthritis.

diminished tibial cartilage volume, and increased tibial bone area [7]. Therefore, determining which modifiable variables are associated with meniscal lesions, even among people with no clinical knee OA, may help to better understand the pathogenesis of knee OA and develop preventative strategies.

Recently, there has been increasing interest in the peak external knee adduction moment in epidemiological studies examining knee joint morphology and the genesis of knee OA and pain [8-11]. The peak external knee adduction moment, which is generated by the combination of the ground reaction force passing medial to the centre of the knee joint during gait and the perpendicular distance of this force from the centre of the knee joint, is a major determinant of 70% of the total knee joint load passing through the medial tibiofemoral compartment during walking [10]. Recently, we demonstrated that the degree of external foot rotation was associated with a reduction in the magnitude of the external peak knee adduction moment during healthy human walking [12]. This result was similar to the previous finding that a toe-out posture of the lower limb also reduced the magnitude of the peak knee adductor moment during late stance [13,14]. Given that the peak external knee adduction moment is a major determinant of the axial load passing through the medial tibiofemoral compartment and that the degree of foot rotation may help to mediate changes in this load, it is possible that these variables may also be associated with the presence of compartment-specific meniscal lesions. The aim of this cross-sectional study was to determine whether the peak external knee adduction moment and the degree of foot rotation occurring during level walking are associated with the presence and severity of meniscal lesions among women with no clinical knee OA.

Materials and methods

Subjects

Twenty women involved in an existing study of healthy aging [8] were recruited through a women's health clinic and advertising in the local media. The study was approved by the ethics committees of Alfred Hospital (Prahran, Victoria, Australia), Caulfield Hospital (Caulfield, Victoria, Australia), and La Trobe University (Melbourne, Victoria, Australia). All participants gave informed consent.

Exclusion criteria were a history of knee OA, radiological OA or any history of symptoms requiring medical treatment, any knee pain for more than 1 day in the month prior to testing, previous or planned knee joint replacement, inflammatory arthritis, malignancy, fracture in the last 10 years, contraindication to MRI (for example, pacemaker, cerebral aneurysm clip, cochlear implant, presence of shrapnel in strategic locations, metal in the eye, and claustrophobia), inability to walk 50 feet without the use of assistive devices, hemiparesis, and any other musculoskeletal, cardiovascular, or neurological condition that would impair normal gait as previously described [8].

Data collection

Weight was measured to the nearest 0.1 kg (shoes, socks, and bulky clothing removed) using a single pair of electronic scales. Height was measured to the nearest 0.1 cm (shoes and socks removed) using a stadiometer. Body mass index (BMI) (weight in kilograms divided by height squared in metres squared) was calculated. A history of knee trauma and knee surgery was obtained.

Magnetic resonance imaging

MRI was performed on the dominant knee (that is, the leg from which a subject stepped off from when initiating walking) as previously described [15]. The following sequence and parameters were used: a T1-weighted fat-suppressed three-dimensional gradient recall acquisition in the steady state; flip angle 55°; repetition time 58 ms; echo time 12 ms; field of view 16 cm; 60 partitions; 512 (frequency direction, superior-inferior) × 512 (phase-encoding direction, anterior-posterior) matrix; one acquisition, time 11 minutes 56 seconds. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31 × 0.31 mm (512 × 512 pixels).

Meniscal tears were assessed in the sagittal view and confirmed in coronal and axial views by experienced radiologists (André Pelletier and Josée Thériault) as previously described [3,7,16]. The presence of a tear was based on the presence of a signal, which was line-shaped, brighter than the dark meniscus, and reached the surface of the meniscus at both ends within six defined regions (anterior horn, body and posterior horn at both medial and lateral tibiofemoral compartments). A semi-quantitative lesion assessment of meniscal tears was also performed. Our scoring system for meniscal damage referred to the accepted MRI nomenclature for meniscal anatomy, which is in accordance with arthroscopic literature [17]. The proportion of the menisci affected by tears was scored separately using the following semi-quantitative scale [3]: 0 = no damage; 1 = one out of three meniscal areas involved (anterior, middle, posterior horns); 2 = two out of three areas involved; 3 = all three areas involved. The intra- and inter-reader correlation coefficients ranged from 0.86 to 0.96 for the meniscal tears [16].

Gait analysis

Gait analyses were conducted in the gait laboratory in the Musculoskeletal Research Centre, La Trobe University. A six-camera Vicon motion analysis system (Oxford Metrics Ltd., Oxford, UK) was used to capture three-dimensional kinematic data during four walking trials on the dominant leg at the subjects' self-selected speed to capture normal gait patterns. Ground reaction forces were measured by a Kistler 9281 force-platform (Kistler Instruments, Winterthur, Switzerland). Inverse dynamic analyses were performed using 'PlugInGait' (Oxford Metrics Ltd.), which is based on a previously proposed model [18], to obtain joint moments calculated about an orthogonal axis system located in the distal segment of a

joint as previously described [8,12]. Inter-ASIS (anterior superior iliac spine) distance was measured using a calliper, allowing the medial-lateral and proximal-distal coordinates of the hip joint centre to be determined by the method previously described [18]. The ASIS to greater-trochanter measurement provided the anterior-posterior coordinate of the hip joint. A knee alignment device (KAD) was used to calculate knee joint axes. The coronal plane of the thigh was defined as the plane containing the hip joint centre, knee marker, and lateral KAD marker. The coronal plane of the shank contained the knee joint centre and lateral malleolus marker. The angle formed by the knee and ankle joint axes measured tibial torsion.

Foot rotation was measured about an axis perpendicular to the foot vector and the ankle flexion axis. It is defined as the angle between the foot vector and the sagittal axis of the shank, projected into the foot transverse plane. This differs from the toe-out angle, which is measured from the long axis of the foot, relative to the line of progression of the body. The foot is defined by the single vector joining the ankle joint centre to the second toe. The relative alignment of this vector and the long axis of the foot is calculated from a static trial using an additional calibration marker from the heel. The foot vector is established by making two rotations about the orthogonal axis. This measure is equal to the angle between the line joining the heel marker and the toe marker, projected in the plane perpendicular to the ankle flexion axis (sagittal). The second rotation is about a foot rotation axis that is perpendicular to the foot vector and the ankle flexion axis. This measure is equal to the angle projected in the plane perpendicular to the foot rotation axis (transverse). The angle is measured between the line joining the heel and toe markers and the line joining the ankle centre and toe marker as previously described [12,19] and according to the protocol stipulated by the Vicon technology in the gait laboratory [20]. Positive values correspond with internal rotation (*Vicon Clinical Manager's User Manual* [20]). Subjects were instructed to walk barefoot at their normal pace over level ground, to capture their natural gait patterns.

Statistical analysis

Gait data were initially examined for normality and linearity. The peak external knee adduction moment and degree of foot rotation occurring when the adductor moment peaked during early and late stance were averaged over four walking trials. Peak external knee adduction moments were normalised to percentage body weight multiplied by height. Linear regression analyses were used to determine the relationship between meniscal tear presence (yes/no) and severity (grade) (independent variables) and peak external knee adduction moments and foot rotation during early and late stance (outcome variables). Age and gender are associated with meniscal tears and also with gait. Our study used restriction to reduce any confounding associated with gender and included age within our multivariate regression analysis. Moreover, since six participants reported a past knee injury, a history of knee injury (yes/no) was also included in the regression analyses. Furthermore, to see whether rotation effects on the menisci were independent of the adductor moment, this was included within the model. Results in which there were P values of less than 0.05 (two-tailed) were considered to be statistically significant. All analyses were performed using SPSS (version 11.0.1; SPSS Inc., Cary, NC, USA).

Results

Meniscal tears were present in the dominant knees of 9 (45%) of the 20 participating women. Six (30%) of these were located medially and 4 (20%) were located laterally. One woman had a meniscal tear in both medial and lateral compartments. Seven of the 20 women had self-reported a knee injury at some time in their life. No injury occurred in the knee that was imaged. All injuries were reported as mild and did not require any treatment. None of these injuries occurred in the knee imaged. There were no significant differences in the prevalence of meniscal tears (medial $P = 1.0$ and lateral $P = 0.7$), peak external knee adduction moments (early and late stance $P = 0.8$), degree of foot rotation when the adductor moment peaked during early ($P = 0.4$) and late ($P = 0.7$) stance, and age ($P = 0.14$) in women who reported a prior injury and those who did not; however, those with a past injury had slightly lower BMIs ($P = 0.04$). Nineteen of the 20 women had a Kellgren-Lawrence (KL) score of 0 whereas one woman had a KL score of 1. The external knee adduction moment peaked at

Table 1

Demographic and biomechanical mean data

	n = 20
Age, years	60.7 (5.5)
Body mass index, kg/m^2	25.3 (4.2)
Kellgren-Lawrence grades, number (percentage)	
Grade 0	19 (95%)
Grade 1	1 (5%)
Prevalence of meniscal tears, number (percentage)	9 (45%)
Prevalence of medial meniscal tears, number (percentage)	6 (30%)
Prevalence of lateral meniscal tears, number (percentage)	4 (20%)
Knee adduction moment[a]	
Early stance	4.0 (0.9)
Late stance	2.2 (0.7)
Foot rotation, degrees[b]	
Early stance	-7.65 (6.0)
Late stance	0.44 (5.6)

Values are presented as mean (standard deviation) unless otherwise stated. [a]Adduction moments are normalised to percentage body weight multiplied by height. [b]Positive values for foot rotation indicate internal rotation and negative values indicate external rotation.

12% (early stance) and 48% (late stance) of the gait cycle. Mean gait, meniscal, and subject data are presented in Table 1.

The peak external knee adduction moment during early stance was positively associated with the presence ($P = 0.04$, $r^2 = 0.3$) and severity ($P = 0.01$, $r^2 = 0.4$) of medial meniscal tears (Table 2). A trend toward significance was also apparent between the presence of medial meniscal tears and the peak external knee adduction moment during late stance ($P = 0.09$) (Table 2). No association between the presence and grade of lateral meniscal tears during either early or late stance and the peak external knee adduction moment was observed. As 7 of the 20 women had a self-reported knee injury in the past, a history of knee injury was included in the model but did not change the association between meniscal tears and the external knee adduction moment (data not shown).

No association between meniscal tears and the degree of foot rotation when the external knee adduction moment peaked during early stance was observed (Table 3). However, the degree of foot rotation when the external knee adduction moment peaked during late stance was positively associated with both the presence ($P = 0.03$, $r^2 = 0.3$) and severity ($P = 0.03$, $r^2 = 0.3$) of medial meniscal tears. The presence of a medial compartment meniscal tear was associated with a 6.2° (95% confidence interval [CI] 0.5 to 11.8; $P = 0.03$) increase in internal foot rotation, and each grade increase in meniscal tear severity was associated with a 3.5° (95% CI 0.35 to 6.6; $P = 0.03$) increase in internal foot rotation (Table 3). When the corresponding peak external knee adduction moment was included in the model, a trend between greater internal foot rotation during late stance and the presence (5.4°, 95% CI -1 to 11.8; $P = 0.09$) and severity (3.0°, 95% CI -0.42 to 6.5; $P = 0.08$) of medial meniscal tears persisted. Moreover, the inclusion of self-report of past history of knee injury in the model did not significantly affect the association between meniscal tears and foot rotation (data not shown).

Discussion

In this cross-sectional study examining women with no clinical knee OA, we have demonstrated that medial meniscal tears are associated with changes in biomechanical factors acting on the medial tibiofemoral compartment during level walking. In particular, the presence and severity of medial meniscal tears were associated with an increased peak external knee adduction moment during early stance and trended toward an association during late stance. Moreover, the presence of medial meniscal lesions was positively associated with the degree of internal foot rotation when the external knee adduction moment peaked during late stance, independent of the magnitude of the adductor moment.

To our knowledge this is the first study to describe a relationship between gait parameters and meniscal tears. We have demonstrated that the presence and severity of medial meniscal tears were positively associated with the peak external knee adduction moment during early stance and trended

Table 2

Association between external peak knee adduction moment during early and late stance and the presence and severity of meniscal tears

	Univariate regression coefficient (95% CI)	*P* value	Multivariate regression coefficient (95% CI)[a]	*P* Value
Early stance				
Any medial meniscal tear y/n[b]	0.8 (-0.1, 1.8)	0.07	1.0 (0.05. 1.9)	0.04
Medial meniscal tear score[c]	0.6 (0.1, 1.1)	0.02	0.6 (0.2, 1.1)	0.01
Any lateral meniscal tear y/n[b]	0.3 (-1.5, 0.8)	0.5	-0.3 (-1.5, 0.9)	0.6
Lateral meniscal tear score[c]	-0.1 (-1.0, 0.7)	0.8	-0.1 (-1.0, 0.7)	0.7
Late stance				
Any medial meniscal tear y/n[b]	0.6 (-0.1, 1.3)	0.09	0.6 (-0.1, 1.4)	0.09
Medial meniscal tear score[c]	0.3 (-0.1, 0.7)	0.13	0.3 (-0.1, 0.7)	0.14
Any lateral meniscal tear y/n[b]	0.2 (-1.1, 0.6)	0.6	-0.2 (-1.1, 0.7)	0.62
Lateral meniscal tear score[c]	-0.07 (-0.7, 0.6)	0.8	-0.07 (-0.7, 0.6)	0.8

[a]Adjusted for age. [b]Increase in peak adduction moment if a meniscal tear is present (tear = 1, no tear = 0). [c]Increase in peak adduction moment for each increase in grade of meniscal tear score. Adduction moments are normalised to percentage body weight multiplied by height. CI, confidence interval.

Table 3

Association between foot rotation during early and late stance and the presence and severity of meniscal tears

	Univariate regression coefficient (95% CI)	*P* value	Multivariate regression coefficient (95% CI)[a]	*P* Value
Early stance				
Any medial meniscal tear y/n[b]	1.7 (-5.1, 8.5)	0.6	0.16 (-5.6, 8.9)	0.6
Medial meniscal tear score[c]	1.1 (-2.7, 4.9)	0.5	1.1 (-3.0, 5.1)	0.6
Any lateral meniscal tear y/n[b]	1.9 (-5.8, 9.6)	0.6	1.9 (-6.2, 9.9)	0.6
Lateral meniscal tear score[c]	1.1 (-4.6, 6.9)	0.7	1.1 (-4.8, 7.1)	0.7
		0.6		
Late stance				
Any medial meniscal tear y/n[b]	6.3 (1.1, 11.6)	0.02	6.2 (0.5, 11.8)	0.03
Medial meniscal tear score[c]	3.6 (0.6, 6.6)	0.02	3.5 (0.35, 6.6)	0.03
Any lateral meniscal tear y/n[b]	2.3 (-4.6, 9.3)	0.5	2.2 (-4.9, 9.3)	0.52
Lateral meniscal tear score[c]	1.0 (-4.2, 6.3)	0.7	1.1 (-4.2, 6.5)	0.6

[a]Adjusted for age. [b]Increase in early stance peak adduction moment if a meniscal tear is present (tear = 1, no tear = 0). [c]Increase in peak adduction moment for each increase in grade of meniscal tear score. Positive foot rotation values indicate internal rotation and negative values represent external rotation. CI, confidence interval.

toward a similar association during late stance. The peak external knee adduction moment is a major determinant of 70% of the total knee joint load passing through the medial tibiofemoral compartment during walking [10], and it is not surprising to have observed these compartment-specific results. Other studies have also demonstrated compartment-specific associations between the peak external adduction moment and other knee joint structures such as the medial tibial plateau area in non-osteoarthritic women [8] as well as medial joint space narrowing in OA populations [11,21] and increased medial compartment cartilage breakdown in rabbits [22].

The presence of medial meniscal tears was also positively associated with the degree of internal foot rotation when the external knee adduction moment peaked during late stance, independent of the magnitude of the adductor moment. We have previously shown that the degree of foot rotation correlates with the knee adduction moment, whereby the magnitude of the peak knee adduction moment during late stance can be reduced by external rotation of the foot [12]. Others have also shown that the magnitude of the toe-out angle (a postural description rather than an isolated joint movement) is inversely associated with the peak external knee adduction moment during late stance [13,14,23]. Therefore, the degree of internal foot rotation during late stance observed in our study may have contributed toward increased medial tibiofemoral joint load by mediating an increase in the peak external knee adduction moment. However, our results demonstrated an association between internal foot rotation and the presence and severity of medial meniscal tears, independent of the peak external knee adduction moment. This suggests that, as well as compressive loads imparted by the knee adduction moment, non-compressive forces such as rotations appear to be an independent determinant of the presence and severity of medial meniscal tears.

This study has demonstrated that gait parameters that isolate medial tibiofemoral joint loads are associated with medial meniscal pathology. It may be that meniscal lesions predict aberrations in gait or alternatively that the gait parameters contributed to the development of these lesions. If the latter were true, our results would imply that by reducing internal foot rotation during late stance, either independent of the knee adductor moment or alternatively by mediating a reduction in the peak external knee adduction moment, meniscal tear prevalence and severity could be reduced. Since meniscal tears are associated with structural changes of OA (including cartilage defect scores, reduced tibial cartilage volume, and increased tibial bone area [2-7,24]), it is possible that modifying the gait parameters examined in this study (for example, via gait retraining or orthoses) may also help to reduce the incidence and burden of knee OA.

The sample size in this study was modest and the range of the 95% CIs was wide, thereby providing the range of uncertainty in our results, however we did have sufficient power to detect a relationship between biomechanical parameters and the presence and severity of meniscal tears. The potential effect of

outliers was also examined and shown not to influence the results, and in many cases the 95% CIs also indicate that the true differences could be quite large (if the upper end of the CI is examined). Furthermore, by selecting only healthy middle-aged women, we were able to reduce the effect of potential confounders such as age and gender. The results of this study, however, are limited to non-osteoarthritic women and therefore are not generalizable to men or osteoarthritic populations. Another potential limitation of this study relates to the biomechanical model we adopted. The axis system that measured the magnitude of knee adduction moment and the degree of foot rotation was calculated from the orientation of the shank. Therefore, the knee adduction moment and foot rotation may not have represented independent variables. However, we previously used this model and showed that the relationship between the peak external knee adduction moment and degree of foot rotation is not consistent across stance [12]. We examined a number of associations within this study, but we did not correct for multiple comparisons as this would have severely reduced our power to detect any effects. While it is possible that the significant findings we observed are a result of chance, this is unlikely as the association between meniscal tears and gait remained consistent regardless of which definition of tear we used. In addition, the significant results observed were biologically plausible. Due to our sample size, the relationship between gait, meniscal tears, and any other potential structural changes in the knee was not explored in this study. Larger longitudinal studies examining this would be required as these relationships may not be simply a matter of confounding but rather structural changes on the causal pathway of biomechanic gait abnormalities and knee disease. In addition, it possible that the associations observed are a result of knee injury rather than altered gait; however, while almost one third of our population reported an injury in their knee at some point during their life, all injuries were reported as mild and did not require any treatment. Anyone with severe injuries or symptoms was excluded. In addition, in women who reported any injury to their knee during their life, their contralateral knee was imaged. To confidently determine that a self-report of knee injury was not confounding our results, a history of knee injury was included within the models and did not alter the results, thus implying that the association between adduction moment, foot rotation, and meniscal tear are independent of knee injury. Finally, because of the cross-sectional nature of this study, we are unable to determine cause and effect and therefore cannot conclude whether gait variables caused meniscal lesions or *vice versa*. Longitudinal studies will be required to determine this.

Conclusion

This study demonstrated a significant positive relationship between the presence and severity of medial meniscal lesions and the magnitude of the peak external knee adduction moment as well as the degree of internal foot rotation during level walking among middle-aged women with no clinical knee OA. Taken together, these results indicate that the presence of medial meniscal tears is associated with changes in biomechanical factors acting on the medial tibiofemoral compartment.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

FC and AW were involved in the design and implementation of the study, including data collection and measurement, and were involved in the analysis and interpretation of data. SD, JM-P, J-PP, GJ, and CD were involved in the design and implementation of the study, including data collection and measurement. MD-T and AT were involved in the analysis and interpretation of data. All authors were involved in the manuscript preparation and read and approved the final manuscript.

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Chapter 5: Local Biomechanical Factors May Explain Different Compartmental Effects

There is a general consensus that one of the leading risk factors for OA is joint biomechanics, including joint geometry which act within the context of a person's systemic susceptibility to OA [76]. Although the disease can occur at any site within the knee joint, the medial compartment is the most common site of tibiofemoral involvement while the lateral compartment is the most common site for patello-femoral involvement [34-38]. Is has been suggested that local biomechanical factors leading to differing compartmental loading may explain these differing effects. Within the tibiofemoral compartment the majority of forces are directed medially [268] and changes in the alignment of the knee dramatically effect joint loading and are associated with both the development and progression of disease [73, 76]. Joint mechanics however are not fixed and it is unclear whether changes in knee alignment are associated with change in knee cartilage volume within the context of disease.

Similarly within the patellofemoral joint, from which the majority of pain emanating from the knee originates, the role of joint biomechanics has received limited attention. The femoral sulcus angle, which forms an articular surface for the patella between the medial and lateral femoral condyles, has been recognised as an important factor in patellofemoral stability [331, 332] and therefore load distribution within the patellofemoral compartment, yet no study has examined the femoral sulcus angle in the context of patellofemoral OA.

The two papers presented within this chapter therefore aim to address these gaps within the literature. The first study explores whether change in static knee angle over two years was associated with a change in tibial knee cartilage in cartilage people with knee OA. We found that a change toward genu valgum reduced the annual rate of medial tibial cartilage volume loss, while change toward genu varum increased the annual rate of medial tibial cartilage volume loss in knee OA. These findings may have important implications for preventing or delaying the progression of medial tibiofemoral OA, and support the rationale behind surgical procedures, such as high tibial osteotomy, used to treat medial compartment knee OA.

5.1 **Davies-Tuck, Miranda** & Teichtahl, Andrew; Wluka AE, Jones G, and Cicuttini FM. Change in knee angle influences the rate of medial tibial cartilage volume loss in knee osteoarthritis. *Osteoarthritis Cartilage* **2009, 17,** 8-11.

The second study investigates the patello-femoral joint and examines whether the femoral sulcus angle is a determinant of compartmental patella cartilage volume. The results of this study showed that the femoral sulcus angle is a cross-sectional determinant of the amount of patella cartilage, but is not a major determinant of the annual change of patella cartilage volume among people with knee OA. These data suggest that a shallower sulcus in the context of established OA may be an advantageous anatomical variant. Further longitudinal studies are required to determine the role of the femoral sulcus angle in OA.

5.2 **Davies-Tuck M,** Teichtahl AJ, Wluka AE, Wang Y, Urquhart DM, Cui J, Cicuttini FM. Femoral sulcus angle and increased patella facet cartilage volume in an osteoarthritic population. **Osteoarthritis Cartilage 2008; 16,** 131-5

PART B: Suggested Declaration for Thesis Chapter

Monash University

Declaration for Thesis Chapter 5: Change in knee angle influences the rate of medial tibial cartilage volume loss in knee osteoarthritis

Declaration by candidate

In the case of Chapter 5, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Literature review, analysis and interpretation of results, manuscript draft preparation	65

The following co-authors contributed to the work. Co-authors who are students at Monash University must also indicate the extent of their contribution in percentage terms:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Andrew Teichtahl	Literature review , interpretation of results, manuscript draft revision	
Anita Wluka	Study design, recruitment of subjects interpretation, data collection, draft revision	
Graeme Jones	Draft revision	
Flavia Cicuttini	Study design, recruitment of subjects, data collection, interpretation, draft revision	

Candidate's Signature		Date

Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
(4) there are no other authors of the publication according to these criteria;

(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
(6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location(s) Department of Epidemiology and Preventive Medicine, Monash University

[Please note that the location(s) must be institutional in nature, and should be indicated here as a department, centre or institute, with specific campus identification where relevant.]

Signature 1

Signature 2

Signature 3

Signature 4

[redacted]

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Osteoarthritis and Cartilage | ICRS International Cartilage Repair Society | OA OSTEOARTHRITIS

Change in knee angle influences the rate of medial tibial cartilage volume loss in knee osteoarthritis

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Summary

Objectives: Identifying factors that influence the rate of cartilage loss at the knee may help to prevent or delay the progression of knee osteoarthritis (OA). Changes in knee alignment alter knee joint load and may affect the rate of cartilage loss. The aim of this study was to determine whether change in knee alignment between baseline and 2 years is associated with a change in knee cartilage volume in knee OA in the subsequent 2.5 years.

Methods: Seventy-eight adults with symptomatic knee OA were recruited using a combined strategy. Radiographs were performed at time 0 and 2 years to determine change in knee alignment, measured on a continuous scale. Magnetic Resonance Imaging was performed at 2 and 4.5 years to determine annual percentage change in medial and lateral tibial cartilage volumes.

Results: In multivariate analyses, for every 1° change toward genu valgum, there is an associated 0.44% reduction in the rate of annual medial tibial cartilage volume loss (95% CI: −0.85%, −0.04%, $P = 0.03$). Similarly, because our measures of change in alignment and cartilage volume were continuous, these results also implied that for every 1° change toward genu varum, there was an associated 0.44% increase in the rate of annual medial tibial cartilage volume loss. Change in knee angle did not significantly affect the rate of loss of the lateral tibial cartilage volume ($P = 0.95$).

Conclusion: Our results have demonstrated that progressive change toward genu valgum reduced the annual rate of medial tibial cartilage volume loss in people with knee OA, without expediting the rate of lateral tibial cartilage volume loss. These findings suggest that methods to reduce varus alignment may delay the progression of medial tibiofemoral OA and warrant further investigation.

Key words: Cartilage, Osteoarthritis, Knee, Alignment, Prevention.

Introduction

Knee osteoarthritis (OA) is a major cause of chronic pain and disability in people aged over 60 years that has profound socioeconomic ramification for both the individual and the healthcare system[1]. Although the disease can occur at any site within the knee joint, the medial compartment is the most common site of tibiofemoral involvement. In established disease, a reduction in cartilage volume signifies disease progression. Despite this, there is a paucity of longitudinal studies examining factors that contribute toward change in cartilage volume. Identifying factors that slow the rate of cartilage loss may help to alleviate the burden of knee OA, and may establish interventions to delay or prevent the need for knee joint replacement.

Static knee alignment is known to influence load distribution at the knee joint. Anatomically, genu varum predates medial tibiofemoral compartment load, while genu valgum predates lateral compartment load[2]. It has been demonstrated that whereas genu varum is associated with medial compartment joint space narrowing and osteophytes, genu valgum is associated with similar changes in the lateral compartment[3]. Recent debate has focused on whether frontal plane alignment is able to predict the development and progression of radiographic knee OA[4,5]. While the general consensus has been that baseline frontal plane malalignment can predict radiographic progression of knee OA[4,5], there have been conflicting reports of whether malalignment predates disease onset. Brouwer and colleagues showed that an increasing degree of varus alignment is associated with both the development and progression of radiographic knee OA[4]. However, Hunter *et al.* found that baseline knee alignment was not associated with incident radiographic knee OA[6]. Reasons for the discrepancies between these two radiographic studies are unclear.

While the aforementioned studies have examined knee alignment and the onset and progression of knee OA radiographically, few studies have directly assessed the

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relationship between joint structures, such as cartilage volume, and knee alignment. To our knowledge, the only study to have used Magnetic Resonance Imaging (MRI) to assess tibial cartilage volume in the context of knee alignment, demonstrated that baseline knee alignment was a determinant of longitudinal cartilage loss in a compartment specific manner among people with knee OA[7]. While this result implies that baseline alignment can predict the progression of cartilage loss, it is unclear whether change in knee alignment is associated with change in knee cartilage volume.

The aim of this longitudinal study was to determine whether change in static knee alignment from baseline to year 2 was associated with a change in knee cartilage in cartilage volume over the subsequent 2.5 years in people with knee OA.

Methods

SUBJECTS

Subjects with symptomatic mild–moderate knee OA were recruited by advertising in local papers, through local general practitioners, rheumatologists and orthopedic surgeons, as well as through the local Arthritis Foundation. All participants in the previous study who had undergone baseline MRI, who were alive, had not received a joint replacement in the study joint and had no contra-indication to MRI (pacemaker, metal implant, claustrophobia, etc.) were eligible and invited to take part in this study. Thus 105 subjects were eligible, since 18 subjects had undergone knee replacement surgery. This study was conducted as part of a larger prospective study whereby radiographic examination was conducted at baseline and 2 years later at the first follow-up. MRI examination was performed at the first and second follow-ups, which were approximately 2.5 years apart. The study was approved by the ethics committee of the Alfred and Caulfield Hospitals in Melbourne, Australia. All subjects gave written informed consent.

Inclusion criteria mandated age over 40 and knee OA according to American College of Rheumatology (ACR) criteria[8]. Subjects were excluded if any other form of arthritis was present, contra-indication to MRI (e.g., pacemaker, cerebral aneurysm clip, cochlear implant, presence of shrapnel in strategic locations, metal in the eye, and claustrophobia), inability to walk 50 feet without the use of assistive devices, hemiparesis of either lower limb, and planned total knee replacement.

RADIOGRAPHS AND DETERMINING STATIC KNEE ALIGNMENT

Each subject attended for a standing anteroposterior (AP) radiograph of their symptomatic knee (or where both knees were symptomatic, the knee with least severe radiographic OA) at baseline and 2 years later at the first radiological follow-up. Lines were drawn through the middle of the femoral shaft and through the middle of the tibial shaft. The angle subtended at the point at which these two lines met in the center of the tibial spines was based on a modification of the method of Moreland[9] validated by Hinman[10]. Knee angles were considered as a continuum ranging from 0 to 360°, with lower values representing increasing varus, and higher values increasing valgus alignment. Intra-observer reliability (expressed as Intraclass Correlation Coefficient) was 0.98[7].

MAGNETIC RESONANCE IMAGING AND THE MEASUREMENT OF CARTILAGE VOLUME

Each subject attended for an MRI of their symptomatic knee at 2 (second radiological follow-up) and 4.5 years. Knee cartilage volume was determined by MRI image processing on an independent workstation using the Osiris software as previously described[11]. Knees were imaged in the sagittal plane on a 1.5-T whole body magnetic resonance unit (Philips) using a commercial transmit–receive extremity coil. The following sequence and parameters were used: a T1-weighted fat suppressed three-dimensional (3D) gradient recall acquisition in the steady state; flip angle 55°; repetition time 58 ms; echo time 12 ms; field of view 16 cm; 60 partitions; 513 × 196 matrix; one acquisition time 11 min 56 s. sagittal images were obtain at a partition thickness of 1.5 mm and an in-plane resolution of 0.31 × 0.83 mm (512 × 196 pixels). The image data were transferred to a workstation.

The volumes of the individual tibial cartilage plates were isolated from the total tibial volume by manually drawing disarticulation contours around the cartilage boundaries on each section. These data were re-sampled by bilinear and cubic interpolation (area of 312 and 312 μm and 1.5 mm thickness, continuous sections) for the final 3D rendering. The volume of the particular tibial cartilage plate was determined by summing the pertinent voxels within the resultant binary volume. A trained observer read each MRI. Independent checks of volume estimates were made in a blinded fashion by a second trained observer[11]. The coefficients of variation (CVs) for the medial and lateral tibial cartilage volumes were 3.4 and 2.0%, respectively. Medial and lateral tibial plateau areas were determined by creating an isotropic volume from the input images which were reformatted in the axial plane. Areas were directly measured from these images. CVs for the medial and lateral tibial plateau areas were 2.3 and 2.4%, respectively[11].

STATISTICAL ANALYSES

Change in cartilage volume and knee alignment was initially assessed for normality before being regressed against each other using linear regression. Known confounders were adjusted for including gender and baseline age and knee angle, and body mass index (BMI) and cartilage volume at 2 years in multivariate analyses. Change in each variable (e.g., knee alignment) was calculated by subtracting follow-up from baseline data. The annual change in each parameter was then calculated by dividing this figure by the time between assessments. Annual percentage change in cartilage volume was obtained by dividing the annual cartilage volume change by the cartilage volume at 2 years and multiplying by 100 to obtain a percentage. All analyses were performed using the SPSS statistical package (standard version 15.0, SPSS, Chicago, IL). A P value of less than 0.05 was considered to be statistically significant.

Results

Of the 105 eligible subjects, 78 (74%) completed the study. Reasons for failure to complete the study included, significant co-morbidity[9], moved interstate[3] and loss to follow-up/refusal to participate[15]. Forty-two (52%) of study participants were female. At study inception, the mean age of the cohort was 63 (±10.5) years, and BMI was 28.2 (±4.6) kg m^{-2}. Characteristics of the study population are shown in Table I.

Our results demonstrated that for every 1° change away from genu varum toward genu valgum, there is an associated 0.44% reduction in the annual rate of medial tibial cartilage volume loss (95% CI: −0.85, −0.04, $P = 0.03$) (see Table II)These results remained significant when change in medial tibial cartilage volume was expressed as annual volume change. Similarly, because our measures of change in alignment and cartilage volume were continuous, these results also implied that for every 1° change toward genu varum, there was an associated 0.44% increase in the rate of annual medial tibial cartilage volume loss. We further analyzed the data by grouping people according to change in a valgus direction (yes/no). After adjustment for confounders, we substantiated a tendency for the annual percentage rate of medial tibial cartilage volume loss to be reduced by 2.0% (95% CI: −4.3%, 0.3%) if the knee angle changed in a valgus direction ($P = 0.08$) (data not shown).

Change in knee angle did not significantly affect the annual rate of lateral tibial cartilage volume loss ($P = 0.95$).

Discussion

We have demonstrated that change in knee alignment from genu varum toward genu valgum from baseline to year 2 was associated with a reduction in the annual rate of medial tibial cartilage volume loss among people with knee OA in the subsequent 2.5 years. Change in alignment did not affect the rate of change in lateral tibial cartilage volume. These findings suggest that methods to reduce varus alignment may delay the progression of medial tibiofemoral OA.

Previously, it was demonstrated that baseline static knee alignment was a determinant of the rate of cartilage loss in

Table I
Characteristics of study subjects

	Total ($n = 78$)
Gender (% female)	52
Age at baseline (years)	63 (10.5)
BMI at baseline (kg m^{-2})	28 (4.7)
Kellgren–Lawrence median grade at baseline (range)	2 (1–3)
WOMAC scores at baseline	
Pain	78.4 (42.6)
Stiffness	36.7 (21.2)
Function	287.4 (161.2)
Total	402.5 (214.4)
Time between baseline and first follow-up (years)	1.9 (0.2)
Time between first follow-up and second follow-up (years)	2.5 (0.4)
Radiographic data	
Knee alignment at baseline (°)	180.5 (5.7)
Knee alignment at follow-up (°)	180.9 (5.8)
Change in knee alignment (first follow-up – baseline) (°)	0.22 (0.35)
Frequency of people whose knees changed in a valgus direction (%)	51.8
Frequency of people whose knees changed in a varus direction (%)	48.2
MRI data	
Tibial cartilage volume at first follow-up (μl)	
Medial	1405 (511)
Lateral	1649 (622)
Tibial cartilage volume at second follow-up (μl)	
Medial	1267 (461)
Lateral	1473 (632)
Annual change (loss) in tibial cartilage volume (μl)	
Medial	56 (85)
Lateral	68 (77)
Annual change (loss) in tibial cartilage volume (%)	
Medial	3.7 (5.9)
Lateral	4.8 (5.6)

Values are reported as mean (±SD) at baseline unless otherwise stated.

More positive knee alignment is oriented away from genu varum.

a compartment specific manner among people with tibiofemoral OA[7]. However, the current study adds to previous knowledge by providing the first evidence that *change* in alignment away from genu varum toward genu valgum is associated with a reduction in the subsequent rate of medial tibial cartilage loss, without increasing the rate of lateral tibial cartilage volume loss in people with knee OA. This infers that minor changes (e.g., 1°) away from genu varum, which ultimately reduces the static load exerted to the medial tibiofemoral compartment, can reduce the rate of cartilage loss in that compartment. This rationale underlies specific surgical procedures, such as high tibial osteotomy, that is used to treat medial compartment knee OA in patients with varus alignment[12], and provides theoretical evidence to support such a surgical approach. However, our study suggests that this can be extrapolated to minor degrees of malalignment.

The mechanism for a reduction in the rate of medial tibial cartilage volume loss with change away from genu varum is likely to be due to complex interactions between biomechanical factors and mechanocellular responses. Cartilage deformation has been demonstrated in response to high impact joint loading[13], and it may be that loading the medial joint increases the rate of medial compartment cartilage loss in the setting of OA. In non-arthritic cartilage, it has been speculated that chondrocytes may respond to mechanical loading by increasing glycosaminoglycan content, causing cartilage "swelling", which may represent a compensatory mechanism to withstand added joint loads[14]. Diseased cartilage may not be equipped with similar compensatory mechanisms, and may, therefore, be more susceptible to the affect of alterations in joint load. Moreover, it may be that in OA joints, aberrations in biomechanics, such as knee alignment, may be either the cause or the result of disease progression.

This study was potentially limited by the relatively small sample and we cannot exclude the possibility that a larger cohort may have demonstrated that change in knee alignment was associated with the rate of cartilage volume loss at the lateral tibia. However, joint load is preferentially directed to the medial compartment of the knee[15] even in the context of genu valgum, and it may be that small deviations toward genu valgum have a greater influence at offsetting medial compartment load than they do at increasing lateral compartment load. Moreover, this study was limited to subjects with established knee OA, and our conclusions are, therefore, generalized to the progression, rather than the onset of disease. It may be that change in knee alignment has no effect on the rate of cartilage volume reduction in non-arthritic states.

We also examined tibial cartilage volume plates only, as these have previously been shown to correlate with femoral cartilage volume both cross-sectionally[16] and longitudinally[17]. Moreover, we examined cartilage volume and not thickness. One of the potential difficulties of measuring cartilage thickness longitudinally is that the defined regions of cartilage have no natural anatomical boundaries and thus assessment of disease will require either co-registration or assessment of images side by side. This is technically challenging and may engender reliability problems. We were also unable to examine the relationship between meniscal damage and bone marrow lesions as these could not be

Table II
The relationship between change in knee alignment and annual percentage change in cartilage volume

	Univariate analysis (95% CI)*	*P*	Multivariate analysis (95% CI)†	*P*
Annual change in medial tibial cartilage volume (μl)	−2.5 (−8.3, 3.3)	0.4	−6.0 (−11.9, −0.15)	0.04
Annual change in medial tibial cartilage volume (%)	−0.24 (−0.6, 0.14)	0.2	−0.44 (−0.95, −0.04)	0.03
Annual change in lateral tibial cartilage volume (μl)	−0.08 (−5.4, 5.3)	0.98	−0.14 (−5.9, 5.6)	0.96
Annual change in lateral tibial cartilage volume (%)	−0.12 (−0.51, 0.27)	0.5	0.01 (−0.4, 0.4)	0.95

*Annual percentage change in tibial cartilage volume (μl or %) for every 1° change in knee alignment.

†Annual percentage change in tibial cartilage volume (μl or %) for every 1° change in knee alignment after adjustment for baseline age, BMI, knee angle, gender and respective cartilage volume and BMI at 2 years.

measured from the MRI sequences employed in this study. Additionally, although we did not obtain full-limb films to assess knee alignment, Hinman *et al.* demonstrated that our method was a valid alternative to full-limb films to predict mechanical axis[10]. We did, however, only examine the association between static knee alignment and knee cartilage, and it is possible that dynamic measures, such as the knee adductor moment, are associated with compartment specific knee cartilage properties. Finally, we did not use a cut-off to categorize subjects as valgus or varus. Although we could have used a correction factor to estimate neutral alignment, this was not an essential component of this study, since our intention was to determine whether change in frontal plane knee alignment (in either a varus or valgus direction) was associated with the rate of compartment cartilage volume loss at the tibia, without exposing subjects to unnecessary ionizing radiation. This approach allowed us to detect associations between small changes in cartilage volume and small changes in knee alignment, independent of whether a person had anatomical genu varum or valgum alignment. This approach also enabled us to conclude that change toward genu valgum was associated with a reduction in the rate of medial tibial cartilage volume loss, without an increase in the rate of lateral tibial cartilage volume loss. Alternatively, our result could be interpreted that change toward genu varum is associated with an increase in the rate of medial tibial cartilage volume loss.

In conclusion, we have demonstrated that change away from genu varum reduced the annual rate of medial tibial cartilage volume loss among people with knee OA. These findings suggest that methods to reduce varus alignment may delay the progression of medial tibiofemoral OA.

Conflict of interest

The authors have no conflict of interest and certify this to be a true and original work.

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PART B: Suggested Declaration for Thesis Chapter

Monash University

Declaration for Thesis Chapter 5: Femoral sulcus angle and increased patella facet cartilage volume in an osteoarthritic population

Declaration by candidate

In the case of Chapter 5, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Literature review, analysis and interpretation of results, manuscript draft preparation + measurement.	65

The following co-authors contributed to the work. Co-authors who are students at Monash University must also indicate the extent of their contribution in percentage terms:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Andrew Teichtahl	Interpretation of results, manuscript draft revision	
Anita Wluka	Study design, recruitment of subjects interpretation, data collection, draft revision	
Yuanyuan Wang	Measurement and draft revision	
Donna Urquhart	Draft revision	
James Cui	Statistical advice and draft revision	
Flavia Cicuttini	Study design, recruitment of subjects, data collection, interpretation, draft revision	

Candidate's Signature		Date

Declaration by co-authors

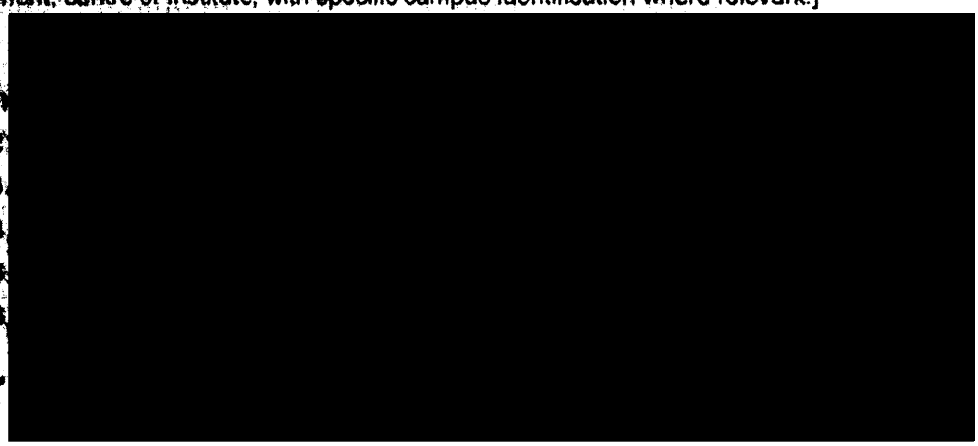
The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.

(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
(4) there are no other authors of the publication according to these criteria;
(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
(6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location(s) Department of Epidemiology and Preventive Medicine, Monash University

[Please note that the location(s) must be institutional in nature, and should be indicated here as a department, centre or institute, with specific campus identification where relevant.]



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Date

Osteoarthritis and Cartilage (2008) **16**, 131–135

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Osteoarthritis and Cartilage | ICRS International Cartilage Repair Society | OA OSTEOARTHRITIS

Brief report

Femoral sulcus angle and increased patella facet cartilage volume in an osteoarthritic population[1]

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Summary

Objective: The patellofemoral joint is an example of an incongruent articulation commonly affected by osteoarthritis (OA). The relationship between femoral sulcus angle and the development and progression of patellofemoral OA is unclear. The aim of this study was to examine the relationship between the femoral sulcus angle at baseline and patella cartilage volume at baseline and at 2-year follow-up among community based adults with established knee OA.

Methods: One hundred subjects had magnetic resonance imaging of their symptomatic knee at baseline and at 2-year follow-up. From these images, patella cartilage volume was determined. Radiographic skyline views of the patellofemoral joint were taken at baseline to measure the femoral sulcus angle.

Results: For every 1° increase in the femoral sulcus angle (i.e., as the sulcus angle became more shallow) there was an associated 9.1 mm^3 (95% CI 3.1, 15.0) increase in medial patella cartilage volume at baseline ($P = 0.003$). There was a similar trend that approached statistical significance between the femoral sulcus angle and the lateral patella facet cartilage volume at baseline ($P = 0.09$). There was no association between the femoral sulcus angle at baseline and the change in patella cartilage volume over 2 years in either patellofemoral compartment.

Conclusion: These results infer that the femoral sulcus angle is a cross-sectional determinant of the amount of patella cartilage, but is not a major determinant of the annual change of patella cartilage volume among people with knee OA. These data suggest that a shallower sulcus in the context of established OA may be an advantageous anatomical variant. Further longitudinal studies are required to determine the role of the femoral sulcus angle in OA.

Key words: Sulcus angle, Patella cartilage, Patella facets, Patellofemoral osteoarthritis.

Introduction

The patellofemoral joint, which is formed by the articulation between the irregularly shaped under-surface of the patella and the femoral trochlear groove, is one example of an incongruent joint that is commonly affected by painful and disabling pathological processes such as osteoarthritis (OA)[1]. Incongruent joints sacrifice stability for mobility, and as a result, are commonly affected by pathological processes. In particular, the larger lateral patellofemoral compartment is most commonly affected by OA[2–4].

Of the bony landmarks along the trochlear groove, the femoral sulcus angle, which forms an articular surface for the patella between the medial and lateral femoral condyles, has been recognised as an important factor in patellofemoral stability[2,5]. Nevertheless, no study has examined the femoral sulcus angle in the context of patellofemoral OA. One reason for this paucity of data may have arisen because of the difficulty in obtaining valid and reliable measures of patellofemoral joint structure, namely cartilage volumes that are sensitive to change. Radiographic quantitation of patellar cartilage, approximated by radiographic joint space, is unreliable when assessed longitudinally by either the skyline or lateral views, since these views may be affected by knee position, patellar tilt and subluxation[6,7]. Moreover, indirect examination of the radiographic joint space width as a surrogate for patellofemoral cartilage has proven to be problematic, unless joint space narrowing is very severe[8]. In contrast, magnetic resonance imaging (MRI) enables patellar cartilage to be directly assessed[9–11]. Another reason may also be that accurate measures of sulcus angle from

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radiographs are problematic due to their dependence on the degree of knee flexion, and only one group has previously described three-dimensional (3D) analysis of the sulcus angle by using an open MRI scanner[12].

The aim of this study was to examine whether the femoral sulcus angle is a determinant of compartmental patella cartilage volume at baseline and change in compartmental volume in a population with OA over 2 years.

Methods

STUDY PARTICIPANTS

Subjects with knee OA were recruited by advertising through local newspapers and the Victorian branch of the Arthritis Foundation of Australia and in collaboration with General Practitioners, Rheumatologists and Orthopaedic Surgeons[13]. Subjects aged over 40 years who fulfilled American College of Rheumatology (ACR) clinical and radiographic criteria for knee OA[14] were examined in this study. Subjects were excluded if any other form of arthritis was present, if there was any contraindication to MRI or if a total knee replacement was planned. The study was approved by the ethics committees of the Alfred and Caulfield hospitals in Melbourne, Australia. All patients gave informed consent.

DATA COLLECTION

At baseline, weight was measured to the nearest 0.1 kg (shoes, socks and bulky clothing removed) using a single pair of electronic scales and height was measured to the nearest 0.1 cm (shoes and socks removed) using a stadiometer. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2).

At baseline, radiographs were taken to determine inclusion in the study. Each subject had a weight-bearing antero-posterior tibiofemoral radiograph, taken in full extension and a skyline (infero-superior) view, taken in the supine position, with 45° of knee flexion (using a perspex positioning wedge). Radiographs were taken of the symptomatic knee. Where both knees had OA and were symptomatic, the knee with least severe radiographic OA was used to reduce subject loss to follow-up for joint replacement surgery. Radiographs were scored independently by two trained observers using a published atlas to classify disease. The radiological features of OA were graded in each compartment (medial tibiofemoral, lateral tibiofemoral and patellofemoral), on a four-point scale (0–3) for individual features of osteophytes and joint space narrowing[15]. In the case of disagreement between observers, the films were reviewed with a third independent observer. Intraobserver reproducibility for agreement on features of OA was 0.93 for osteophytes (grade 0,1 vs 2,3) and 0.93 for joint space narrowing (grade 0,1 vs 2,3). Interobserver reproducibility was 0.86 for osteophytes and 0.85 for joint space narrowing (κ statistic)[16].

RADIOGRAPHY AND FEMORAL SULCUS ANGLE DETERMINATION

Radiographic skyline (infero-superior) views were taken with each subject positioned in supine with 45° of knee flexion (using a perspex positioning wedge). Femoral sulcus angles were measured from these images. The femoral sulcus angle was measured independently by two trained observers as previously described[17,18]. The femoral sulcus angle was defined by lines joining the highest points of the medial and lateral condyles and the lowest point of the intercondylar sulcus (Fig. 1). The angle was measured using the software program Osiris (University of Geneva, Switzerland). All angles were reported in degrees. The intraclass correlation coefficient (ICC) between the two sulcus angle measurements was calculated to assess the reliability of the two sulcus angle measurements using Stata software version 9 (StataCorp 2005). The ICC was estimated to be 0.98 (95% CI 0.97–0.99).

MRI

Each subject had an MRI performed at baseline and approximately 2 years later on the same knee was X-rayed at baseline. Knees were imaged in a sagittal plane on the same 1.5-T whole-body magnetic resonance unit (Signa Advantage HiSpeed GE Medical Systems, Milwaukee, WI, USA) using a commercial receive-only extremity coil. The following sequence and parameters were used: a T1-weighted, fat-suppressed 3D gradient recall acquisition in the steady state; flip angle 55°; repetition time 58 ms; echo time 12 ms; field of view 16 cm; 60 partitions; 512 (frequency direction, superior–inferior) × 512 (phase encoding direction, anterior–posterior) matrix; one acquisition,

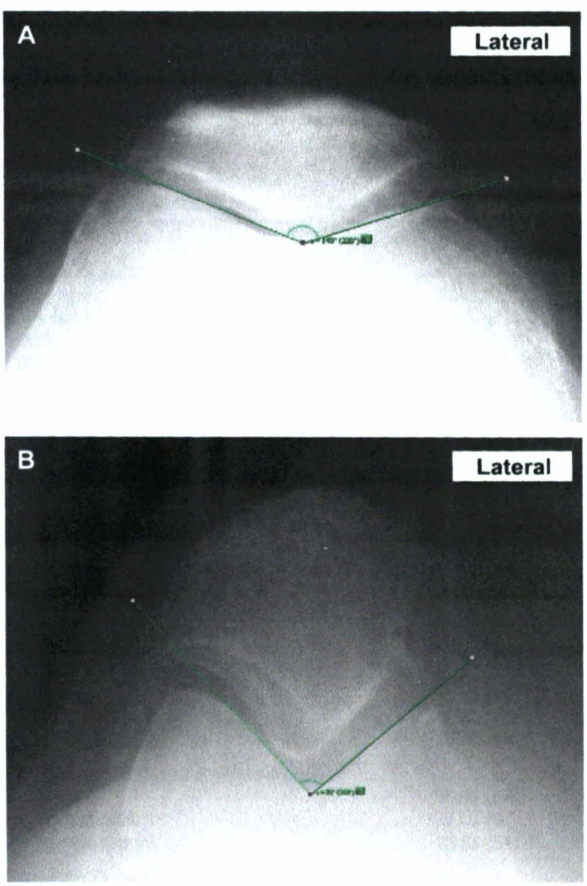

Fig. 1. Skyline radiographs used to measure femoral sulcus angle. The femoral sulcus angle was defined by lines joining the highest points of the medial and lateral condyles and the lowest point of the intercondylar sulcus as previously described[17,18]. (A) Shows a shallow and (B) a narrow sulcus angle.

time 11 min 56 s. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31 × 0.31 mm (512 × 512 pixels)[9,16].

Patella cartilage volume was determined at baseline and approximately 2 years later by means of image processing in an independent workstation using the software program Osiris (University of Geneva, Switzerland) by creating an isotropic volume from the input images, which were reformatted in the axial plane (voxel dimensions 0.427 × 0.427 × 1.281 mm^3) using a validated method[16,19]. Briefly, we used the patella ridge to divide the patella cartilage into medial and lateral facets. Medial and lateral patella facet cartilage volumes were then measured separately by two trained observers on each MRI by manually drawing disarticulation contours around the cartilage boundaries on each section blind of patient's identification and study sequences as previously described[20] (Fig. 2). The coefficient of variation (CV) for cartilage volume measures was 2.6% for patellar cartilage.

STATISTICAL ANALYSES

Change in knee cartilage volume was obtained by subtracting cartilage volume at follow-up from that at baseline. The annual change was calculated by dividing this figure by the time between MRI scans. The annual change in cartilage volume followed a normal distribution and therefore the association between femoral sulcus angle and change in cartilage volume was explored using multiple linear regression. The contribution of each of the variables to the models was determined by examining the total and partial variance of each of the variables on the patella cartilage volume[21]. A *P*-value of less than 0.05 (two-tailed) was regarded as statistically significant. All analyses were performed using the SPSS statistical package (standard version 15.0, SPSS, Chicago, IL, USA).

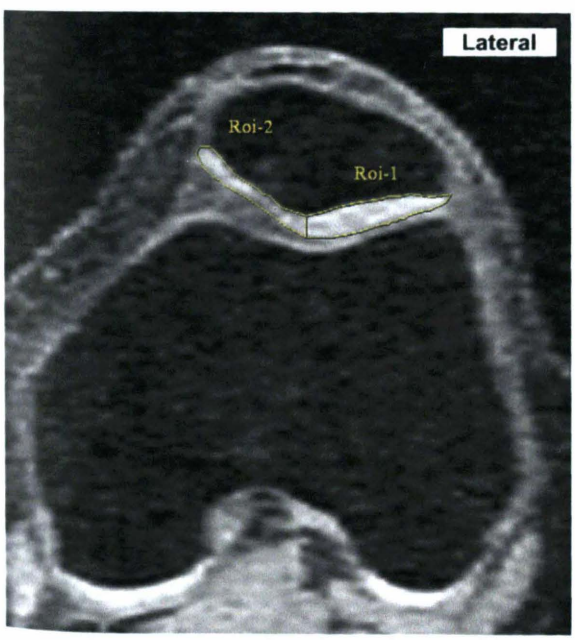

Fig. 2. Patella cartilage volume was determined by means of image processing using the software program Osiris (University of Geneva, Switzerland). The patella ridge was used to divide the patella cartilage into medial and lateral facets. Medial and lateral patella facet cartilage volumes were then measured separately on each MRI by manually drawing disarticulation contours around the cartilage boundaries on each section.

Results

Femoral sulcus angle and patella facet measurements were available for 100 participants. The characteristics of the study population are presented in Table I. The mean (standard deviation (SD)) femoral sulcus angle was 131.8° (10.2°). There was no significant difference in the femoral sulcus angle between men and women ($P = 0.9$). Within the patellofemoral compartment grade 2 or more patellofemoral osteophytes were present in 35% and grade 2 or more patellofemoral joint space narrowing was observed in 12% of participants. Within the medial tibiofemoral compartment, 20% of participants had grade 2 or greater tibiofemoral osteophytes and 27% had grade 2 or greater tibiofemoral joint space narrowing. Within the lateral tibiofemoral compartment, 31% of participants had grade 2 or greater tibiofemoral osteophytes and 11% had grade 2 or greater tibiofemoral joint space narrowing.

In cross-sectional analyses, for every 1° increase in the femoral sulcus angle (i.e., as the femoral sulcus became more shallow), there was a 9.1 mm^3 (95% CI 3.1, 15.0) increase in the medial ($P = 0.003$) and a 15.4 mm^3 (95% CI 1.8, 29) increase in total patella cartilage ($P = 0.03$) volume after adjustment for potential confounders (age, gender, BMI and patella bone volume). A similar trend between femoral sulcus angle and the lateral patella cartilage volume at baseline was also observed ($P = 0.09$). No significant relationship between femoral sulcus angle and annual

Table I
Characteristics of study population

Subject characteristics	Total eligible ($n = 100$)
Age (years)	63.3 (10.2)
Gender (% female)*	61 (61%)
Height (cm)	166.7 (8.9)
Weight (kg)	81 (15.4)
BMI (kg/m^2)	29 (4.9)
Femoral sulcus angle (°)	131.8 (10.2)
Patellofemoral osteophytes ≥grade 2*	35 (35%)
Patellofemoral joint space narrowing >grade 2*	12 (12%)
Tibiofemoral osteophytes ≥grade 2*	
Medial compartment	20 (20%)
Lateral compartment	27 (27%)
Tibiofemoral joint space narrowing ≥grade 2*	
Medial compartment	31 (31%)
Lateral compartment	11 (11%)
Patella bone volume (mm^3)	21,064 (4840)
Patella cartilage volume at baseline (mm^3)	
Total	2535 (968)
Medial compartment	972 (408)
Lateral compartment	1563 (592)
Patella cartilage volume at 2 years (mm^3)	
Total	2085 (848)
Medial compartment	779 (371)
Lateral compartment	1313 (539)
Annual change in patella cartilage volume (mm^3)	
Total	231 (236)
Medial compartment	102 (103)
Lateral compartment	128 (148)

All variables described as mean (±SD) unless otherwise stated.
*Described as total number (percentage).

change in cartilage volume over the study period was observed (Table II).

Discussion

The cross-sectional component of this study demonstrated that for every 1° increase in the femoral sulcus angle (i.e., as the sulcus became more shallow), there was an associated 9.1 mm^3 increase in medial patella cartilage volume among people with knee OA. A similar trend was also seen between the femoral sulcus angle and the lateral patella cartilage volume. There was no significant relationship between the femoral sulcus angle at baseline and longitudinal change in patella cartilage volume over 2 years.

No previous study has examined the relationship between the femoral sulcus angle and patella cartilage volume. The femoral sulcus has previously been examined in the context of patellofemoral subluxation/dislocation[17,22,23], trochlear dysplasia[24] and patellofemoral pain syndrome[18]. In a small study that examined 21 women with recurrent patellar dislocation, it was shown that the mean femoral sulcus angle was significantly larger (shallower) in people with recurrent dislocation compared to a control group with no history or signs of dislocation[23]. Another small study of 16 patients with femoral trochlear dysplasia showed that the femoral trochlear groove was significantly shallower compared to 23 people without trochlear dysplasia[24]. In a study comparing 23 women with patellofemoral pain to 12 control subjects, although the femoral sulcus angle was not significantly different between the two groups, a more shallow sulcus was associated with increased lateral patella displacement and tilt[18], which are both thought to contribute towards the pathogenesis of patellofemoral pain.

Although the existing literature has generally supported the notion that a shallower femoral sulcus is associated with decreased patellofemoral congruency and stability[2,5], our study is the first to have examined the femoral sulcus angle in the context of knee OA. Whereas our cross-sectional results demonstrate that a greater medial patella cartilage volume is associated with a shallower femoral sulcus in people with knee OA, the femoral sulcus angle was not a significant determinant of the annual patella cartilage loss. Further studies are required to determine factors influencing the rate of loss of patella cartilage volume. Mechanistically, it may be that a shallower femoral sulcus increases the surface area for articulation with the patella. Increased articular contact area theoretically may reduce contact pressure and thus allow better distribution of retropatellar joint load[25]. Therefore, a shallower rather than a deeper femoral sulcus may be better suited to providing optimal mechanical stimulation to articular cartilage and reducing contact stresses. Moreover, the tendency for the association between the femoral sulcus angle and patella cartilage volume to be significant in the medial patellofemoral compartment may signify that a shallower sulcus may be a determinant that protects the medial compartment from degenerative processes.

The results of this study are limited to people with knee OA and cannot be generalised to non-osteoarthritic populations. Although we demonstrated a cross-sectional association between the femoral sulcus angle and patella cartilage volume, the baseline angle was not significantly associated with change in cartilage volume over 2 years. It may be that our sample size or follow-up period was too small to detect change in cartilage volume that is attributed to the sulcus angle. In cross-sectional analyses, the sulcus angle explained only 8% of the 53% variance in patella cartilage after multiple regression analyses. Although significant, this relatively small contribution towards patella cartilage volume variability may infer that longer time frames are needed to detect significant changes in cartilage volume that are related to the femoral sulcus angle. Another limitation of our study is that the two-dimensional analysis of the femoral sulcus angle employed is highly dependent on the degree of knee flexion, however, a method for accurately measuring the sulcus angle (bony or cartilaginous) from MR images is yet to be validated. Patella tilting and lateralisation were also not measured in this study and may contribute to changes in the patella cartilage.

In conclusion, this study demonstrated that among people with knee OA, a more shallow sulcus is associated with increased medial patella cartilage volume compared to a deeper sulcus angle. Despite this cross-sectional association, the femoral sulcus angle at baseline was not associated with longitudinal change in patella cartilage volume over 2 years. These data suggest that a shallower sulcus in the context of established OA may be an advantageous anatomical variant. Further longitudinal studies are required to elucidate the role the femoral sulcus angle plays in OA.

Table II
The relationship between femoral sulcus angle and patella cartilage volume

	Univariate analysis		Multivariate analysis	
	Regression coefficient (95% CI)*	*P*-value	Regression coefficient (95% CI)	*P*-value
Baseline cartilage volume				
Total patella cartilage	14.6 (−5.4, 35)	0.15	15.4 (1.8, 29)	**0.03**
Medial patella cartilage	8.1 (−0.002, 16.14)	**0.05**	9.1 (3.1, 15)†	**0.003**
Lateral patella cartilage	6.4 (−5.5, 18.4)	0.3	7.2 (−1.2, 15.5)†	0.09
Annual change in cartilage volume				
Total patella cartilage	2.2 (−2.8, 7.2)	0.4	0.6 (5.2, 4)	0.8
Medial patella cartilage	0.81 (−1.4, 2.9)	0.46	−0.16 (−2.3, 1.9)‡	0.9
Lateral patella cartilage	1.4 (−1.7, 4.5)	0.4	−0.3 (−3.1, 2.5)‡	0.84

The bold values signify that the findings were statistically significant.

*Change in dependent variable per unit increase in sulcus angle.

†Change in patella cartilage volume (mm^3) per unit increase in sulcus angle in the regression equations after adjustment for age, gender, BMI and a patella bone volume (mm^3).

‡Annual change in patella cartilage volume (mm^3) per unit increase in sulcus angle without and with adjustment for age, gender, BMI, patella bone volume and baseline cartilage volume.

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Chapter 6: The Natural History and Significance of Bone Marrow Lesions

There is increasing interest on the role of bone marrow lesions (BMLs), detected by magnetic resonance imaging (MRI), in the pathogenesis of knee osteoarthritis (OA)[41, 78]. Histological examination of BMLs in knees has shown that they may represent areas of osteonecrosis, oedema, trabecular abnormalities and bony remodeling [170]. While BMLs have been shown to be present in both symptomatic [41, 181, 270, 271] and asymptomatic populations [171, 173], most previous studies have focussed on symptomatic populations with established knee OA [41, 172, 174-177, 239, 270, 278]. In these populations BMLs are associated with knee symptoms [41, 172, 174-177] and progression of disease [78, 175, 178, 270, 277] and once present they are unlikely to resolve [239, 270, 271, 278].

There is very little information however, about the role of BMLs in healthy asymptomatic populations. The presence of BMLs at baseline in these populations has been shown to be associated with longitudinal progression of cartilage defects and loss of cartilage volume [333] suggesting that BMLs also have a pathogenic role in pre-clinical OA however, there is no data on the natural history of BMLs in asymptomatic populations or of factors that may affect the development of BMLs. In addition the relationship between incident BMLs and the resolution of BMLs prevalent at baseline and change in knee cartilage over time has not been described.

The two papers presented within this chapter therefore describe the natural history and significance of bone marrow lesions in those with no clinical knee OA.

The first paper describes the natural history of BMLs in an asymptomatic population. It presents the rates of incidence of BMLs over two years and factors related to the incidence including the development of pain symptoms. It also reports the natural history of BMLs present at baseline and factors associated with either their resolution or persistence. We found that the rate of incidence of BMLs is lower than previously described in populations with OA. Incident BMLs were associated with increased BMI and the development of pain. Approximately half the BMLs present at baseline

resolved. These data suggest that in pain free people with no clinical knee OA, BMLs are reversible and may provide a target for interventions aimed at the prevention of knee OA.

6.1 **Davies-Tuck ML**, Wluka AE, Wang Y, English DR, Giles GG, and Cicuttini FM. The Natural History of Bone Marrow Lesions in Community Based Adults with no clinical knee osteoarthritis. **Annals of the Rheumatic Diseases, 68**(6):904-8

The second paper describes the relationships between the development or resolution of BMLs and knee cartilage properties in a 2 year prospective study of asymptomatic middle-aged adults. In this study we found that the development of new BMLs was associated with progressive knee cartilage pathology while resolution of BMLs prevalent at baseline was associated with reduced progression of cartilage pathology. These observations suggest that BMLs may provide an important target for the prevention of knee OA.

6.1 **Davies-Tuck ML**, Wluka A, Forbes A, Wang Y, English DR, Giles GG & Cicuttini F Development of Bone Marrow Lesions is associated with adverse effects on knee cartilage while resolution is associated with improvement-a potential target for prevention of knee osteoarthritis: a longitudinal study. **Arthritis Research and Therapy 12: R10**

PART B: Suggested Declaration for Thesis Chapter

Monash University

Declaration for Thesis Chapter 6: The Natural History of Bone Marrow Lesions in Community Based Adults with no clinical knee osteoarthritis.

Declaration by candidate

In the case of Chapter 6, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Literature review, measurement of bone marrow lesions, analysis and interpretation of results, manuscript draft preparation	70

The following co-authors contributed to the work. Co-authors who are students at Monash University must also indicate the extent of their contribution in percentage terms:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Anita Wluka	Study design, recruitment of subjects interpretation, data collection, draft revision	
Yuanyuan Wang	Measurement of cartilage and draft revision	
Dallas English	Draft revision	
Graham Giles	Draft revision	
Flavia Cicuttini	Study design, recruitment of subjects, data collection, interpretation, draft revision	

Candidate's Signature		Date

Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;

(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
(4) there are no other authors of the publication according to these criteria;
(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
(6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location(s) Department of Epidemiology and Preventive Medicine, Monash University

[Please note that the location(s) must be institutional in nature, and should be indicated here as a department, centre or institute, with specific campus identification where relevant.]

Signature 1

Signature 2

Signature 3

Signature 4

Signature 5

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The natural history of bone marrow lesions in community-based adults with no clinical knee osteoarthritis

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ABSTRACT

Objective: Although bone marrow lesions (BML) have been implicated in the pathogenesis of osteoarthritis, their natural history in a healthy population is unknown. This study in a healthy, pain-free population aimed to examine the natural history of BML; factors associated with incidence and progression of BML over 2 years and whether incident BML are associated with the development of pain.

Methods: 271 subjects with no clinical knee osteoarthritis, being pain free at baseline, underwent magnetic resonance imaging of their dominant knee at baseline and 2 years later. The presence of BML was assessed.

Results: In knees initially free of BML, incident BML developed in 14% of people over the study period. Increased body mass index (BMI; odds ratio (OR) 1.15, 95% CI 1.06 to 1.2, p = 0.001) was associated with incident BML. Those who developed a BML were more likely to develop knee pain compared with those in whom no BML developed (OR 4.2, 95% CI 1.2 to 15.1, p = 0.03). Among those in whom BML were present at baseline, 46% completely resolved. There was no association between age, gender and BMI and persistence of BML over 2 years.

Conclusion: In this healthy population, the rate of incident BML is lower than previously described in a population with osteoarthritis. Incident BML are associated with increased BMI and the development of pain. Approximately half the BML present at baseline resolved. These data suggest that in pain-free people with no clinical knee osteoarthritis, BML are reversible and may provide a target for interventions aimed at the prevention of knee osteoarthritis.

Bone marrow lesions (BML), detected by magnetic resonance imaging (MRI) have been implicated in the pathogenesis of knee osteoarthritis.[1–3] They have been shown to be present in both symptomatic[2–5] and asymptomatic populations.[6 7] The presence of BML has been associated with structural changes in the knee, including joint space narrowing,[8] loss of cartilage[1 4 9] and increased prevalence and severity of cartilage defects.[6 7 10] Although BML have been associated with knee pain, the data are conflicting, whereas a number of studies report a relationship between BML and pain,[2 9 11] others show no such association.[3 4 12 13] Both mechanical factors such as such as trauma,[14–19] knee malalignment,[3] and increased weight[6] as well as systemic factors such as osteoprotective medications[20] and nutritional factors[21 22] have been shown to affect the risk of BML.

Little is known about the natural history of BML. Most previous studies have focussed on symptomatic populations with established clinical knee osteoarthritis rather than asymptomatic populations.[4 5 13 15 23] In subjects with symptomatic knee osteoarthritis, the data suggest that BML are unlikely to resolve, with one study suggesting that 99% of BML either remained the same or increased over 15 or 30 months[5] and another finding that only 10% resolved over 2 years.[15] Similarly, in a population with chronic knee pain, which included subjects with and without radiographic osteoarthritis, only 22% completely resolved over 2 years.[23]

There are no data on the natural history of BML in asymptomatic populations with no clinical knee osteoarthritis or of factors that may effect the development of BML. The aim of this paper was to examine the natural history of BML in a healthy population, free of knee pain at baseline, and to identify factors associated with the incidence and progression of BML over 2 years and whether incident BML are associated with the development of pain.

METHODS

Subjects

Subjects were recruited from the Melbourne Collaborative Cohort Study, a prospective cohort study of community-based people, aged 40–69 years, established to examine the role of lifestyle and genetic factors in the risk of cancer and chronic diseases from middle age and beyond, as described.[7] Subjects were excluded if they had: osteoarthritis, as defined by the American College of Rheumatology clinical criteria;[24] current or past knee disease; a history of knee pain in the past 5 years lasting for more than 24 h; a previous knee injury requiring non-weight-bearing treatment for more than 24 h or surgery (including arthroscopy); or a history of any arthritis diagnosed by a medical practitioner or contraindication to MRI, as described.[25] The study was approved by the Human Research Ethics Committee of the Cancer Council of Victoria and Monash University Standing Committee on Ethics in Research Involving Humans. All participants gave written informed consent.

Data collection

Study participants completed a questionnaire that included information on their demographics at baseline and at the 2-year follow-up. Weight was measured to the nearest 0.1 kg (shoes, socks and

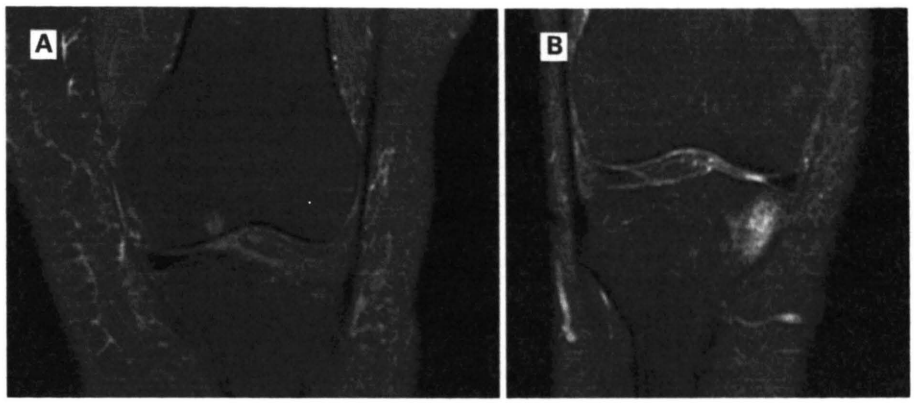

Figure 1 Coronal T2 images of a "large" (A) and "very large" (B) bone marrow lesion.

bulky clothing removed) using a single pair of electronic scales. Height was measured to the nearest 0.1 cm (shoes and socks removed) using a stadiometer. From these data, body mass index (BMI; weight/height kg/m^2) was calculated. The development of pain in the knee was assessed at the 2-year follow-up by asking the question "have you had any pain in your knee in the past 12 months, yes or no?", for those who answered yes, the duration of pain was also determined by asking whether this pain lasted for "less than 24 h", "more than 24 h but less than half a month" or "more than half a month" in the past 12 months.

Magnetic resonance imaging

An MRI of the dominant knee of each subject (defined as the lower limb from which the subject stepped off when initiating gait) was performed at baseline and approximately 2 years later.[6] Knees were imaged in the coronal plane on a 1.5-T whole-body magnetic resonance unit (Philips Medical Systems, Eindhoven, The Netherlands) using a commercial transmit–receive extremity coil. The following sequence and parameters were used: fat saturated, fast spin echo three-dimensional, T2-weighted (2200 ms, 20/80 ms/90° repetition time/echo time/flip angle) with a slice thickness of 3 mm, a 0.3 interslice gap, one excitation, a field of view of 11–12 cm and a matrix of 256 × 128 pixels.[6]

Bone marrow lesions

Assessment of BML

BML were defined as areas of increased signal intensity adjacent to subcortical bone present in either the medial or lateral, distal femur or proximal tibia assessed on coronal T2-weighted fat-saturated images.[26] Two trained observers (MD and AW), who were blinded to patient characteristics, as well as the sequence of images, together assessed the presence of lesions for each subject. The presence or absence of a BML was determined. A lesion was defined as "large" if it appeared on two or more adjacent slices and encompassed at least one quarter of the width of the tibial or femoral cartilage being examined from coronal images. This is comparable to the previously described "grade 2" BML by Felson *et al*.[2] Lesions were further classified as "very large" if they appeared on three or more slices. This is comparable to the previously described "grade 3" by Felson *et al*.[2] Fig 1 shows a "large" (A) and "very large" (B) BML. The reproducibility for determination of the BML was assessed using 60 randomly selected knee MRI (κ value 0.88, $p<0.001$). If a person had more than one BML underlying a cartilage plate, the BML of the highest grade was used for analysis.

Statistical analysis

Descriptive statistics for the characteristics of the study subjects were tabulated. Independent sample t tests were performed to compare means and χ^2 analysis to compare proportions. The relationship between risk factors, incident pain and incident BML was assessed by binary logistic regression. A p value of less than 0.05 (two-tailed) was regarded as statistically significant. All analyses were performed using the SPSS statistical package (standard version 14.0).

RESULTS

Of the 297 pain-free subjects imaged at baseline, 271 (90%) participants completed the 2-year follow-up. The reasons for the loss to follow-up of 26 subjects were: death (three); withdrawal for health reasons (four); withdrawal of consent (10); ineligible for follow-up (pacemakers) (four) and inability to

Table 1 Characteristics of study subjects

	No BML present in knee at baseline (n = 234)			BML present in knee at baseline (n = 37)		
	Incident BML (n = 33)	No incident BML (n = 201)	p Value	BML resolved (n = 17)	BML persisted (n = 20)	p Value
Age (years)	57.7 (5.8)	57.7 (5.0)	0.9	57.8 (6.4)	58.6 (5.5)	0.7
Female (%)	23 (69%)	121 (60%)	0.3*	11 (65%)	13 (65%)	0.98
Height (cm)	167 (9.4)	168 (9.1)	0.6	170 (10.6)	169 (9.1)	0.5
Weight (kg)	78 (17)	72 (13)	0.02	72.6 (10.6)	74.3 (13.4)	0.7
BMI (kg/m^2)	27.9 (5.2)	25.4 (3.7)	0.01	25.2 (4.6)	26.1 (4.0)	0.5
Incident knee pain at follow-up	15 (45%)	57 (29%)	0.05*	5 (29%)	8 (40%)	0.5*

Values are reported as mean (SD) except where otherwise noted. p Value indicates the difference between subjects. *Pearson χ^2 for dichotomous variables. BMI, body mass index; BML, bone marrow lesion.

Table 2 Risk factors for incident BML in the knee

	Univariate analysis* (OR, 95% CI)	p Value	Multivariate analysis (OR, 95% CI)	p Value
"Large" or "very large" BML				
Age at baseline (years)	0.99 (0.93 to 1.1)	0.9	1.0 (0.9 to 1.1)†	0.7
Gender (female vs male)	1.5 (0.7 to 3.3)	0.3	1.66 (0.7 to 3.8)‡	0.2
BMI at baseline (kg/m^2)	1.15 (1.0 to 1.2)	0.002	1.15 (1.06 to 1.2)§	0.001
"Very large" BML				
Age at baseline (years)	0.97 (0.9 to 1.1)	0.6	0.99 (0.9 to 1.1)†	0.99
Gender (female vs male)	0.98 (0.3 to 3.1)	0.97	0.9 (0.3 to 3.1)‡	0.9
BMI at baseline (kg/m^2)	1.24 (1.1 to 1.4)	<0.001	1.2 (1.1 to 1.4)§	0.001

*Odds ratio (OR) for an incident bone marrow lesion (BML) at follow-up per unit increase in respective variable. †OR for an incident BML at follow up per 1-year increase in age adjusted for gender and body mass index (BMI). ‡OR for an incident BML at follow-up when female = 1 adjusted for age and BMI. §OR for an incident BML at follow-up per unit increase in BMI adjusted for age and gender.

be contacted (five). The only significant difference between those who completed follow-up and those who were lost to follow-up was that those lost to follow-up were slightly heavier ($p = 0.01$). All participants were pain free at baseline.

Incidence of BML

A total of 234 of the 271 participants did not have a BML at baseline. Over 2 years, 33 knees developed new BML (14% of knees). Those who developed a BML weighed more ($p = 0.02$), had a higher BMI ($p = 0.01$) and reported a higher proportion of incident pain ($p = 0.05$) (table 1). Of the 33 BML that developed over the study, 20 (61%) were located within the medial and 13 (39%) within the lateral compartment. One person developed a BML in both the medial and lateral compartments. Of the 33 incident BML, 13 were graded as "very large".

Factors associated with developing BML over 2 years are presented in table 2. Although age, gender and height were not significantly associated with developing a BML over 2 years, increased BMI was significantly associated with developing a BML both before ($p = 0.002$) and after ($p = 0.001$) adjusting for confounders. For every unit increase in BMI, there was an associated 15% increased likelihood of developing a BML ($p = 0.001$) and a 20% increased likelihood of developing a "very large" BML ($p = 0.001$), after adjusting for confounders.

We examined whether self-reported knee pain at follow-up differed in those subjects who developed a BML over 2 years compared with those who did not (table 3). Among people with no BML in the knee at baseline, incident knee pain was reported in 45% of people who developed a BML compared with 29% who remained BML free. Self-reported knee pain, assessed by the question "have you had any pain in your knee in the past 12 months, yes or no?", was positively associated with incident BML over 2 years, with people who developed a "very large" BML being 4.2 (95% CI 1.2 to 15.1) times more likely to report knee pain than people who did not develop a "very large" BML, after adjusting for confounders ($p = 0.03$). Furthermore, developing an incident BML over 2 years was positively associated with the development of knee pain lasting more than half a month (OR 3.5, 95% CI 1.21 to 10.0, $p = 0.02$) in univariate analyses. The development of a "very large" incident BML was also significantly associated with the development of knee pain lasting more than half a month in the past 12 months in univariate analysis (OR 6.5, 95% CI 1.8 to 23.9, $p = 0.005$) and after adjusting for confounders (OR 5.0, 95% CI 1.11 to 23.3, $p = 0.04$).

Natural history of prevalent BML

Thirty-seven of the 271 participants who completed follow-up had a BML at baseline. Twenty-five (67%) BML were located within the medial and 14 (38%) within the lateral compartment. Two people had a BML in both compartments. Of the 37 BML that were present at baseline, 11 were graded as "very large", with six located within the medial and five within the lateral compartment. Of the 37 BML that were present at baseline, 20 (54%) persisted at follow-up and 17 (46%) completely resolved. There were no significant differences in age ($p = 0.7$), gender ($p = 0.98$) and BMI ($p = 0.50$) between people who had a BML at baseline that resolved compared with people whose BML persisted over the 2-year study period (table 1). Furthermore, there were no significant associations between risk factors such as age ($p = 0.8$), gender ($p = 0.98$), weight ($p = 0.4$) and BMI ($p = 0.6$) and BML persisting over 2 years. There were no associations with the development of pain.

Table 3 Association between incident BML and development of pain at follow-up

	Univariate analysis* (OR, 95% CI)	p Value	Multivariate analysis (OR, 95% CI)	p Value
Incident BML				
Self-reported knee pain	2.1 (0.99 to 4.5)	0.05	1.9 (0.9 to 4.3)†	0.10
Pain in knee for over half a month in past 12 months	3.5 (1.21 to 10.0)	0.02	2.53 (0.8 to 7.9)‡	0.11
Incident "very large" BML				
Self-reported knee pain	3.9 (1.2 to 12.4)	0.02	4.2 (1.2 to 15.1)†	0.03
Pain in knee for over half a month in past 12 months	6.5 (1.8 to 23.9)	0.005	5.01 (1.11 to 23.3)‡	0.04

*Odds ratio (OR) for having self-reported knee pain if a there was an incident bone marrow lesion (BML) compared with if there was not. †OR for having self-reported knee pain if there was an incident BML compared with if there was not, adjusted for age, gender and body mass index (BMI). ‡OR for have pain in the knee for more than half a month in the past 12 months compared with if not, adjusted for age, gender and BMI.

DISCUSSION

In this population of healthy participants with no clinical knee osteoarthritis, BML developed in 14% of people over 2 years. Increased BMI was a risk factor for incident BML, and incident BML were associated with the development of knee pain in a population in which all participants were free of pain at the beginning of the study. Approximately half of the BML present at baseline resolved over the 2-year study period.

This is the first study to report the natural history of BML and risk factors associated with the incidence of BML in a healthy, pain-free population. Incident BML have previously been reported in cohorts mixed in relation to radiographic osteoarthritis diagnosis and/or pain symptoms.[5 15 23] In a population with chronic knee pain, in which 80% had radiographic knee osteoarthritis, new lesions developed in 21% of people over 2 years.[15] Similarly, among patients with primary knee osteoarthritis, new BML developed over 15 or 30 months in 20% of knees.[5] In a small study of 47 people with painful knees with (88%) or without (12%) osteoarthritis, eight new BML were identified over 2 years.[23] Our finding that BML developed in 14% of people over 2 years is lower than has been described in symptomatic populations. Furthermore, approximately half the BML present at baseline resolved over 2 years, which is higher than has previously been shown in subjects with osteoarthritis and/or knee pain.[4 5 15 23] These data suggest that, not only are BML less common in asymptomatic subjects with no clinical knee osteoarthritis, but they are more likely to resolve and the rate of development is lower.

In this study, we found that incident BML were associated with developing knee pain. The association between pain and BML is conflicting.[3 4 14 15] To our knowledge only one previous study has reported an association between incident BML and incident pain and this was in a population of people who either had knee osteoarthritis or were at high risk of developing osteoarthritis.[13] In that study, incident BML were found to be more common in the knees of people with incident pain over 15 months compared with those with no incident knee pain.[13] This current study has extended these findings by observing a consistent relationship between incident BML and the development of pain in a healthy, middle-aged population. While subchondral bone has been suggested as a contributor to knee pain,[27] the exact causes of knee pain remain unclear. It is known that the marrow of subchondral bone is richly innervated with nociceptive pain fibres.[28] Taken together with the findings of our study, this suggests that the role of bone in knee pain may be partly mediated via BML development.

We found that increased BMI was a risk factor for incident BML. This has not previously been examined in a longitudinal study. However, these findings are consistent with data from a cross-sectional study of asymptomatic middle-aged women, which showed that the prevalence of BML was associated with increased body weight and BMI.[6] A similar association was also found in a cross-sectional study examining a population containing people with and without radiographic osteoarthritis.[10]

The findings of this study suggest that BML occur in healthy populations but are less common and are more likely to resolve compared with osteoarthritis/symptomatic populations.[9] In this current study, the incidence of BML was also associated with developing new knee pain, mirroring previous observations in osteoarthritis/symptomatic populations. This suggests that BML play a role in knee pain in osteoarthritis possibly as a continuum from a normal to a clinically diseased joint. Furthermore, in this study obesity was associated with developing new BML. Both mechanical factors, such as knee malalignment[8] and increased weight,[6] have been shown to be risk factors for BML prevalence. Knee alignment was not examined in this study and it may be that, in part, the effect of obesity as a risk factor for BML acts via malalignment. However, systemic factors such as nutritional factors[21 22] also affect the risk of BML so a metabolic effect of obesity may also be possible. Given that BML have also been shown to be associated with structural changes associated with the progression of osteoarthritis,[1 4–9] it may be that by preventing their development in healthy populations it could help to reduce the incidence and burden of knee pain.

This study has a number of potential limitations. First, the study examined a healthy, community-based population selected on the criteria of no knee pain or injury, and therefore the results are not generalisable to symptomatic populations or people who have injured their knees. However, the findings of our study can be generalised to populations that may be targeted for primary prevention. Second, we did not obtain radiographs of the knees, so that subjects may have had asymptomatic, radiographic osteoarthritis. However, we used the American College of Rheumatology clinical criteria of osteoarthritis to determine the status of knees, and individuals with significant knee injury in the past, pain at baseline, knee surgery or a medical diagnosis of any other type of arthritis were excluded.[24] It is possible that the findings reported of a lower rate of development and persistence compared with symptomatic/radiographic osteoarthritis populations reflect the low prevalence of undetected radiographic osteoarthritis that may be present in this population. However, it could be argued that the development of radiological osteoarthritis is a step along the spectrum of changes from the normal to the osteoarthritis knee. BML incidence and/or persistence may represent one such step in the pathway because it has been shown that 10% of knee cartilage is lost by the time the first radiological changes of osteoarthritis can be identified.[29] Finally, due to the small number of prevalent BML at baseline, our analysis of persistent BML may not have been sufficiently powered to detect any weak associations between age, gender, BMI and symptoms.

The findings of this study suggest that BML develop in healthy populations at a lower rate than has been reported in osteoarthritis populations, and that approximately half of the BML present at baseline resolved. Increased weight and BMI were risk factors for incident BML. Furthermore, the incidence of BML was positively associated with the development of pain. These data suggest that in middle-aged people with no clinical knee osteoarthritis, BML are reversible and may provide a target for interventions aimed at symptoms and the prevention of knee osteoarthritis.

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Competing interests: None.

Ethics approval: The study was approved by the Human Research Ethics Committee of the Cancer Council of Victoria and Monash University Standing Committee on Ethics in Research Involving Humans.

Patient consent: Obtained.

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PART B: Suggested Declaration for Thesis Chapter

Monash University

Declaration for Thesis Chapter 6: Incident Bone Marrow Lesions are associated with increased cartilage loss, progression of defects and bone expansion over 2 years in Community Based Individuals with No Clinical Knee Osteoarthritis

Declaration by candidate

In the case of Chapter 6, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Literature review, measurement of bone marrow lesions, analysis and interpretation of results, manuscript draft preparation	70

The following co-authors contributed to the work. Co-authors who are students at Monash University must also indicate the extent of their contribution in percentage terms:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Anita Wluka	Study design, recruitment of subjects interpretation, data collection, draft revision	
Andrew Forbes	Statistical advice, draft revision	
Yuanyuan Wang	Measurement of cartilage and draft revision	
Dallas English	Draft revision	
Graham Giles	Draft revision	
Flavia Cicuttini	Study design, recruitment of subjects, data collection, interpretation, draft revision	

Candidate's Signature		Date

Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
(4) there are no other authors of the publication according to these criteria;
(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
(6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location(s) Department of Epidemiology and Preventive Medicine, Monash University

[Please note that the location(s) must be institutional in nature, and should be indicated here as a department, centre or institute, with specific campus identification where relevant.]

Signature 1

Signature 2

Signature 3

Signature 4

Signature 5

Signature 6

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(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
(4) there are no other authors of the publication according to these criteria;
(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
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Signature 1

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RESEARCH ARTICLE Open Access

Development of bone marrow lesions is associated with adverse effects on knee cartilage while resolution is associated with improvement - a potential target for prevention of knee osteoarthritis: a longitudinal study

Miranda L Davies-Tuck[1], Anita E Wluka[1,2], Andrew Forbes[1], Yuanyuan Wang[1], Dallas R English[3,4], Graham G Giles[3], Richard O'Sullivan[5] and Flavia M Cicuttini*[1]

Abstract

Introduction: To examine the relationship between development or resolution of bone marrow lesions (BMLs) and knee cartilage properties in a 2 year prospective study of asymptomatic middle-aged adults.

Methods: 271 adults recruited from the Melbourne Collaborative Cohort Study, underwent a magnetic resonance imaging scan (MRI) of their dominant knee at baseline and again approximately 2 years later. Cartilage volume, cartilage defects and BMLs were determined at both time points.

Results: Among 234 subjects free of BMLs at baseline, 33 developed BMLs over 2 years. The incidence of BMLs was associated with progression of tibiofemoral cartilage defects (OR 2.63 (95% CI 0.93, 7.44), $P = 0.07$ for medial compartment; OR 3.13 (95% CI 1.01, 9.68), $P = 0.048$ for lateral compartment). Among 37 subjects with BMLs at baseline, 17 resolved. Resolution of BMLs was associated with reduced annual loss of medial tibial cartilage volume (regression coefficient -35.9 (95%CI -65, -6.82), $P = 0.02$) and a trend for reduced progression of medial tibiofemoral cartilage defects (OR 0.2 (95% CI 0.04, 1.09), $P = 0.06$).

Conclusions: In this cohort study of asymptomatic middle-aged adults the development of new BMLs was associated with progressive knee cartilage pathology while resolution of BMLs prevalent at baseline was associated with reduced progression of cartilage pathology. Further work examining the relationship between changes and BML and cartilage may provide another important target for the prevention of knee osteoarthritis.

Introduction

There is increasing interest in the role of bone marrow lesions (BMLs), detected by magnetic resonance imaging (MRI), in the pathogenesis of knee osteoarthritis (OA) [1,2]. Histological examination of BMLs in knees has reported that they may represent areas of osteonecrosis, oedema, trabecular abnormalities and bony remodeling [3]. BMLs are present in both symptomatic [4-7] and asymptomatic populations [8,9]. Although BMLs are found to be extremely common in OA populations and, once present, are unlikely to resolve [7,10,11], in asymptomatic populations they tend to have a more fluctuating course [12]. BMLs have most commonly been described in relation to mechanical factors such as trauma [13-16], knee malalignment [17], and increased body weight [8]. However, more recently systemic factors such as osteo-protective medications [18] and nutritional factors [19,20] have been reported to affect the risk of BMLs.

Very little is known about the relation between BMLs and other changes in knee structures in asymptomatic, clinically healthy populations. Most previous studies have focussed on symptomatic populations with established knee OA

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[6,10,11,21], where BMLs are associated with knee symptoms [4,21-25] and progression of structural changes including joint space narrowing [17], loss of cartilage [6,26] and increased prevalence and severity of cartilage defects [23,27]. More recently in an asymptomatic population, the presence of BMLs at baseline was shown to be associated with longitudinal progression of cartilage defects and loss of cartilage volume [28] suggesting that BMLs also have a pathogenic role in pre-clinical OA.

The significance of development or resolution of prevalent BMLs has only recently been examined in populations with, or at high risk of, knee OA [6,7,29]. In two of these studies, the majority of BMLs persisted so both could only examine the effect of change in size of the BMLs, had limited ability to examine incident BMLs, and had no power to investigate resolution [6,7]. In contrast, for participants of the Multi-centre Osteoarthritis Study (MOST) who either had or were at high risk of OA, approximately 40% of BMLs completely resolved and about one-third of cartilage locations developed new BMLs over 30 months, but no significant association between resolution of BMLs and change in cartilage was seen. In addition, the presence, resolution and progression of the BMLs was observed simultaneously within the same knee suggesting that complete resolution of all BMLs in a knee occurred less frequently. Worsening of BMLs and development of new BMLs was associated with increased cartilage loss compared with where BMLs remained stable [29]; however, no comparison between knees with incident BMLs and knees that remained free of BMLs was made. Recently, we have shown for asymptomatic populations that BMLs fluctuate with about 50% resolving and about 14% of people developing new ones over two years [12,30]. Thus, the aim of this study was to examine the relation between incident BMLs and the resolution of BMLs prevalent at baseline and change in knee cartilage over two years in a cohort of asymptomatic middle-aged adults.

Materials and methods

Participants

The study was conducted within the Melbourne Collaborative Cohort Study, a prospective cohort study of 41,528 people, assembled to examine the role of lifestyle and genetic factors in the risk of cancer and chronic diseases in Melbourne, Australia [31]. Participants for the current study were recruited from this cohort in 2003-04 if they were aged between 50 and 79 years without any of the following exclusion criteria: a clinical diagnosis of knee OA as defined by American College of Rheumatology criteria [32]; knee pain lasting for more than 24 hours in the past five years; a previous knee injury requiring non-weight bearing treatment for more than 24 hours or surgery (including arthroscopy); a history of any form of arthritis diagnosed by a medical practitioner or a contraindication to MRI, as previously described [33]. The study was approved by The Cancer Council Victoria's Human Research Ethics Committee and the Standing Committee on Ethics in Research Involving Humans of Monash University, Melbourne. All participants gave written informed consent.

Anthropometric data

Height (cm) was measured using a stadiometer with shoes removed at baseline (1990-94). Weight (kg) was measured with bulky clothing removed at the time of MRI. Body mass index (BMI) was calculated from these data (weight (kg)/height^2 (m^2)).

MRI and the measurement of BMLs, cartilage volume and defects

MRI

An MRI of the dominant knee (defined as the lower limb from which the subject stepped off from when initiating gait) for each participant was performed between October 2003 and December 2004 and approximately two years later, as described on a 1.5-T whole body MR unit (Philips, Medical Systems, Eindhoven, the Netherlands) using a commercial transmit-receive extremity coil [9]. The following sequences and parameters were used: fat suppressed, gradient recall acquisition in the steady state, three dimensional T1-weighted (58 msec/12 msec/55°, repetition time/echo time/flip angle), one signal average, slice thickness 1.5 mm, field of view 16 cm and matrix 512 × 512 scans. In addition, a coronal T2-weighted fat-saturated acquisition, (3500 to 3800 msec/20/80 msec/90°, repetition time/echo time/flip angle), two signal averages, echo train length of 10, with a slice thickness of 3.0 mm, a 1.0 inter slice gap, 1 excitation, a field of view of 13 cm, and a matrix of 256 × 192 pixels was also obtained [8].

Assessment of BMLs

BMLs were defined as areas of ill-defined increased signal intensity adjacent to subcortical bone present in either the medial or lateral, distal femur or proximal tibia assessed of coronal T2-weighted fat-saturated images [34]. Two trained observers (MD and AW), who were blinded to patient characteristics, as well as sequence of images, together assessed the presence of lesions for each subject. The presence or absence of a BML was determined as previously described [34]. Two trained observers, who were blinded to patient characteristics, as well as sequence of images, together assessed the presence of lesions for each subject. The presence or absence of a BML was determined as previously described [17,28]. Briefly, a lesion was defined as present if it appeared on two or more adjacent slices underlying the cartilage plate. A BML was defined as 'incident' if it was present at follow up in the knees without BMLs at baseline. A BML was defined as 'resolved' if it was present at baseline but disappeared at follow up. A BML was classified as

'persistent' if it was present in the same location on both the baseline and follow-up scans. The reproducibility for determination of the BMLs was assessed using 60 randomly selected knee MRIs (κ value 0.88, $P < 0.001$).

Measurement of cartilage volume

The volumes of individual cartilage plates (medial and lateral tibia) were measured from the total volume by manually drawing disarticulation contours around the cartilage boundaries on each section on a workstation as described [33]. The coefficients of variation for the medial and lateral tibial cartilage volume measures were 3.4% and 2.0% respectively [35,36]. Annual change in cartilage volume was calculated as follow up cartilage volume subtracted from initial cartilage volume then divided by the period of time between MRI scans, as described [35].

Assessment of cartilage defects

Cartilage defects were graded on the sagittal T1-weighted MR images with a classification system as previously described [37-39], in the medial and lateral tibial and femoral cartilages. Cartilage defects were graded as follows: grade 0, normal cartilage; grade 1, focal blistering and intracartilaginous low-signal intensity area with an intact surface and bottom; grade 2, irregularities on the surface or bottom and loss of thickness of less than 50%; grade 3, deep ulceration with loss of thickness of more than 50%; grade 4, full-thickness cartilage wear with exposure of subchondral bone. A cartilage defect also had to be present in at least two consecutive slices. The baseline and follow-up cartilage defects were graded in duplicate (the cartilage defects were re-graded one month later), unpaired and blinded to the sequence. The defect scores at medial tibiofemoral (0-8) and lateral tibiofemoral (0-8) compartments were used in the study. Intra-observer reliability (expressed as intraclass correlation coefficient, ICC) was 0.90 for the medial tibiofemoral compartment and 0.89 for the lateral tibiofemoral compartment [40]. Change in cartilage defects in a compartment was classified as to whether or not they progressed (i.e. increase in cartilage defect score), regressed (i.e. reduction in cartilage defect score) or remained stable (i.e. no change in cartilage defect score).

Table 1: Characteristics of participants

	With BMLs at baseline (n = 37)			Free of BMLs at baseline (n = 234)		
	BMLs persisted (n = 20)	**BMLs resolved (n = 17)**	***P* value**	**BMLs developed (n = 33)**	**No BMLs developed (n = 201)**	***P* value**
Age (years)	58.6 (5.5)	57.8 (6.4)	0.70[1]	57.7 (5.9)	57.8 (5.0)	0.90[1]
Gender (% female)	13 (65%)	11 (65%)	0.98[2]	23 (70%)	122 (61%)	0.30[2]
Body mass index (kg/m²)	25.9 (3.9)	24.8 (4.1)	0.50[1]	28.0 (5.1)	25.4 (3.7)	0.01[1]
Annual change in cartilage volume (μl)						
Medial tibial	36.0 (39.2)	10.5 (45.9)	0.13[1]	34.0 (54.6)	19.5 (50.0)	0.08[1]
Lateral tibial	25.6 (67.2)	28.4 (42.1)	0.08[1]	37.6 (57.0)	21.0 (48.4)	0.88[1]
Progression of tibiofemoral cartilage defects, number (%)						
Medial	7 (35%)	3 (18%)	0.15[2]	11 (33%)	44 (21%)	0.24[2]
Lateral	9 (18%)	8 (47%)	0.008[2]	15 (45%)	47 (23%)	0.90[2]

Mean (standard deviation) unless otherwise stated. BML = bone marrow lesion.

[1] Independent samples t-test

[2] Chi-squared test

Table 2: Relation between compartment specific incident bone marrow lesions and longitudinal change in knee cartilage (n = 234)

	Univariate analysis regression coefficient/odds ratio(95% CI)	P value	Multivariate analysis regression coefficient/odds ratio (95% CI)*	P value
Medial compartment				
Annual change in cartilage volume	4.12 (-19.30, 27.60)	0.73	2.37 (-21.78, 26.53)[1]	0.85
Cartilage defects progress vs no change	1.86 (0.70, 4.93)	0.21	2.63 (0.93, 7.44)[2]	0.07
Lateral compartment				
Annual change in cartilage volume	21.2 (-5.86, 48.20)	0.12	18.04 (-9.72, 45.80)[1]	0.2
Cartilage defects progress vs no change	3.0 (1.01, 8.93)	0.05	3.13 (1.01, 9.68)[2]	0.05

[1] Annual change in tibial cartilage volume if an incident bone marrow lesion (BML) developed compared with if no BML developed after adjusting for age, gender, body mass index (BMI) and respective baseline tibial plateau area

[2] Odds ratio for cartilage defects to progress if an incident BML developed compared with if no BML developed after adjusting for age, gender, BMI and respective baseline cartilage volume

CI = confidence interval.

Statistical analysis

All variables were assessed for normality by visually inspecting histograms. Baseline characteristics for the 271 subjects who completed both MRI scans were tabulated. Linear regression was used to examine the compartment specific relation between having an incident or resolved BML and annual change in cartilage volume. Logistic regression was used to determine the compartment specific odds of cartilage defect progression versus regression/stability in relation to if a person had an incident BML or a resolved BML over two years. Potential confounders of age, gender, BMI, and tibial plateau area for annual change in cartilage volume were included in multivariate analyses. A P value less than 0.05 (two-tailed) was regarded as statistically significant. All analyses were performed using the SPSS statistical package (version 15.0.0, SPSS, Cary, NC, USA).

Results

Two hundred and seventy-one (90%) of the originally recruited 297 participants completed both MRI scans at baseline and approximately two years later. Reasons for loss to follow up included: death (3), withdrawal for health reasons (4), withdrawal of consent (10), ineligible for follow up (pacemakers) (4), and inability to be contacted (5). The only significant difference between those who completed follow up and those who were lost to follow up was that those lost to follow up were slightly heavier ($P = 0.01$). Of the 271 participants, 234 (86%) did not have a BML in their knee at baseline. Over the two-year study period, 33 (14%) developed a BML in their knee. Of the 37 (14%) participants who had a BML in their knee at baseline, 20 (54%) persisted and 17 (46%) resolved over the two-year study period. The characteristics of the participants are presented in Table 1.

Relation between incident BMLs and tibiofemoral cartilage properties

The associations between developing an incident BML and annual change in cartilage volume and progression of tibiofemoral cartilage defects are presented in Table 2. Within the medial compartment developing an incident BML was not associated with annual change in medial cartilage volume, but a trend for incidence of medial BMLs being associated with progression of medial tibiofemoral cartilage defects was observed (odds ratio (OR) = 2.63, 95% confidence interval (CI) = 0.93 to 7.44, $P = 0.07$). A similar finding was seen in the lateral compartment. Although incidence of lateral BMLs was not associated with annual change in lateral cartilage volume, having an incident lateral BML was associated with a 3.13 fold (95% CI = 1.01 to 9.68, $P = 0.05$) increased odds of having lateral tibiofemoral defects progress. Figure 1 shows MRI images of knee that developed an incident BML over the two-year period and the worsening of a tibial defect located above

the incident BML.

Relation between resolved BMLs and tibiofemoral cartilage properties

The compartment specific associations between having a BML resolve compared with it persisting over two years and annual change in cartilage volume and progression of tibiofemoral defects are presented in Table 3. Having a medial BML resolve over the study period was associated with a trend for reduced annual loss in medial tibial cartilage volume (regression coefficient = -28.7 μl, 95% CI = -58.11 to 0.68, $P = 0.05$) in univariate analyses. After adjusting for potential confounders this relation became significant (regression coefficient = -35.9 μl, 95% CI = -65 to -6.82, $P = 0.02$). A trend for resolution of medial BMLs and reduced likelihood of progression of medial tibiofemoral defects was also observed in both univariate (OR = 0.23, 95% CI = 0.05 to 1.08, $P = 0.06$) and multivariate analyses (OR = 0.2, 95% CI = 0.04 to 1.09, $P = 0.06$). No relation between the resolution of lateral BMLs and annual change in lateral cartilage volume or progression of lateral tibiofemoral defects was seen.

Discussion

In this cohort of asymptomatic middle-aged adults, the development of new BMLs in knees free of BMLs at baseline was associated with the progression of tibiofemoral cartilage defects over two years. In contrast, the resolution of BMLs was associated with reduced loss of medial tibial cartilage volume and a trend towards reduced progression of tibiofemoral cartilage defects.

The relation between incident BMLs and change in cartilage has only recently been examined [29]. Among elderly participants with or at high risk of knee OA, development of new BMLs was associated with a worsening cartilage score as assessed using the WORMS (Whole organ MRI score) scale compared with knees where a BML remained stable; however, a comparison of cartilage loss with knees that remained BML free was not performed [29]. Although we did not show a relation between incident BMLs and change in cartilage volume, there was progression of cartilage defects. This may be due to the relative short duration of two years of follow up; in a pain-free population, people are likely to have slower cartilage loss, and also due to the fact that cartilage defects are an earlier and independent marker of cartilage pathology [37]. We have shown that cartilage defects are present in asymptomatic people with no clinical or radiological OA and to be predictors of cartilage loss in healthy people [41] and those with OA [37], independent of initial cartilage volume. Thus, it may be that the relation we have observed between incident BMLs and cartilage defects reflects early cartilage pathology and longer duration of follow up may be needed in order to observe subsequent cartilage volume loss.

In this asymptomatic population we found that the resolution of BMLs over two years was associated with beneficial effects on cartilage as evidenced by reduced loss of tibial cartilage volume and a trend towards reduced progression of tibiofemoral cartilage defects, suggesting this is not sim-

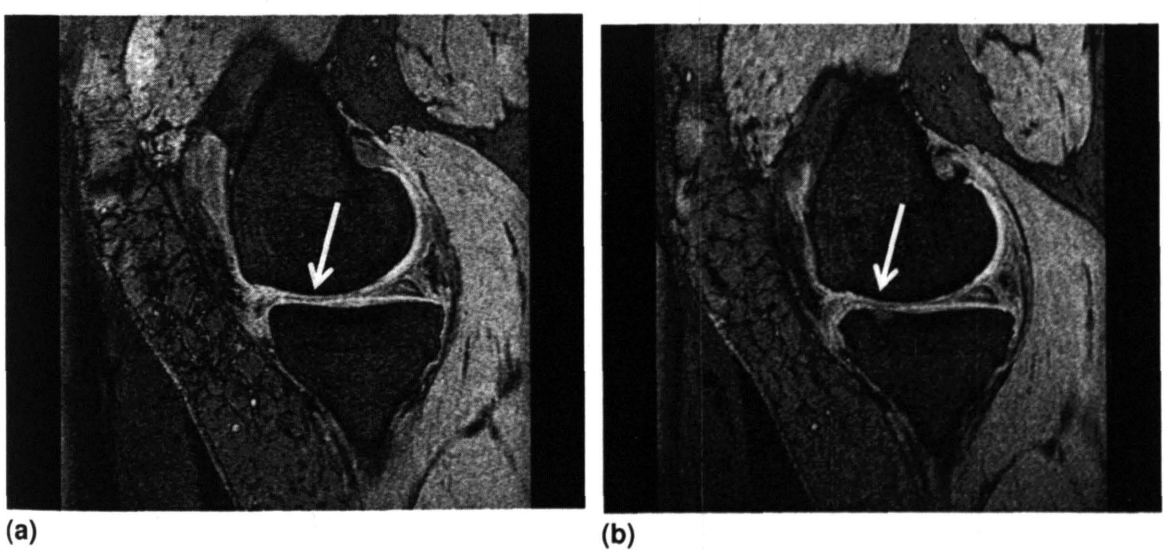

Figure 1 Magnetic resonance images. **(a)** Magnetic resonance image of knee showing no bone marrow lesion (BML) and a grade 2 medial tibial defect at baseline. **(b)** Magnetic resonance image showing an incident medial tibial BML and a grade 3 medial tibial defect above the BML at follow up.

Table 3: Relation between compartment specific resolution compared with persistence of bone marrow lesions and change in knee cartilage (n = 37)

	Univariate analysis regression coefficient/odds ratio (95% CI)	*P* value	Multivariate analysis regression coefficient/odds ratio (95% CI)*	*P* value
Medial compartment				
Annual change in cartilage volume	-28.70 (-58.11, 0.68)	0.05	-35.90 (-65.00, -6.82)[1]	0.02
Cartilage defects progress vs no change	0.23 (0.05, 1.08)	0.06	0.20 (0.04, 1.09)[2]	0.06
Lateral compartment				
Annual change in cartilage volume	24.70 (-18.88, 68.37)	0.26	23.41 (-23.13, 70)[1]	0.31
Cartilage defects progress vs no change	1.08 (0.24, 4.90)	0.92	1.08 (0.22, 5.39)[2]	0.92

[1] Annual change in tibial cartilage volume if a bone marrow lesion (BML) resolved vs persisted after adjusting for age, gender, body mass index (BMI) and respective baseline tibial plateau area

[2] Odds ratio for cartilage defects to progress if a BML resolved vs persisted after adjusting for age, gender, BMI and baseline cartilage volume
CI = confidence interval.

ply due to cartilage swelling. Our results are supported by recent observations in OA populations [6,7,29]. For subjects with OA, an increase in size of BML was shown to be associated with increasing C-terminal cross-linking telopeptide of collagen type II levels [6] and increased cartilage loss [7,29]. To our knowledge only one study, the MOST, has examined cartilage changes in knees where BMLs resolved; however, no significant association was observed between resolution of BMLs and change in cartilage [29]. This may, at least in part, be due to the mixed nature of the MOST population because the purpose of the MOST was to examine a population at high risk of OA. In the MOST population, approximately 12% had symptomatic OA, approximately 24% had symptoms and about one-third had a Kellgren Lawrence score greater than or equal to two and past injury and surgery were not excluded. Therefore, the joints of these participants may already be further along the pathological pathway of structural change from the normal joint to one with OA, where the factors culminating in a BML, and acting on the whole knee, are established. In this situation, the reduction in change of cartilage associated with the resolution of BMLs may be lessened. In contrast, our population was asymptomatic and participants with prior injury or knee surgery were excluded.

There is growing evidence to suggest that BMLs have an important role in the pathogenesis of knee OA. They are common and persistent in symptomatic OA where they are associated with pain and progression of OA [4,6,17,21-26]. Although less common in asymptomatic people, BMLs are also associated with progressive knee cartilage pathology [28,42]. In this asymptomatic population with no clinical OA, the development of new BMLs was associated with adverse effects on knee cartilage, while resolution of BMLs was associated with improvement in cartilage. Although it has been suggested that BMLs are largely due to adverse biomechanical factors, we, and other investigators, have shown that systemic factors also affect the risk of BMLs [18,20,43]. It may be that in the observed relation between BMLs and cartilage, factors contributing to the development of BMLs have resulted in impairment of the supply of nutrients and oxygen to the overlying cartilage plate, which may also reduce the strength of the bony support of articular cartilage [44,45]. Our data also suggest that this is reversible because resolution of BMLs was associated with reduction in cartilage defects and cartilage loss. Thus identifying factors that reduce the incidence of BMLs and increase their resolution may offer therapeutic targets in the prevention of knee OA.

This study has a number of potential limitations. Firstly, it examined a healthy asymptomatic population selected on the criteria of no knee pain or injury and therefore, the results are not generalisable to symptomatic populations or people who have injured their knees. On the other hand, the findings from our study can be generalised to populations that may be targeted for primary prevention or early treatment of knee OA. Second, we did not obtain radiographs of the knees, so some subjects may have had asymptomatic radiographic OA. However, we used the American College

of Rheumatology clinical criteria of OA [32] to determine the status of knees, and individuals with significant knee injury in the past, pain at baseline, knee surgery or medical diagnosis of any other type of arthritis were excluded. Due to the small number of persistent BMLs we were unable to examine change in BML size. The small number of BMLs may have also reduced our power to detect significant associations and may explain the trends reported. In this study we did not assess knee alignment, which has been shown to be associated with the presence of BMLs [17]. If malalignment were to be a major determinant of BMLs, we would not expect it to change significantly in a healthy asymptomatic population over a period of only two years, so would expect it to underestimate the relations we observed.

Conclusions

In this cohort study of asymptomatic middle-aged adults the development of new BMLs was associated with progressive knee cartilage pathology, while resolution of BMLs prevalent at baseline was associated with reduced progression of cartilage pathology. Further work examining the relation between changes and BML and cartilage may provide another important target for the prevention of knee OA.

Abbreviations

BMI: body mass index; BML: bone marrow lesion; CI: confidence interval; CTX-II: C-terminal crosslinking telopeptide of collagen type II; MOST: Multi-centre Osteoarthritis Study; MRI: magnetic resonance imaging; OA: osteoarthritis; OR: odds ratio.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

FC, AW, DE, GG and RO were all involved in the design and implementation of the study including data collection and measurement. MD, AE, AF, YY and FC were involved in the analysis and interpretation of the data. All authors were involved in the manuscript preparation.

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Chapter 7: Systemic Risk Factors for Bone Marrow Lesions

Given the significance of bone marrow lesions and their role in knee OA, understanding modifiable factors which may influence their development is important. While originally thought to predominantly be a result of joint mal-alignment [78] there is increasing evidence to suggest that they may also be associated with systemic or vascular factors [279, 289, 290]. The ends of long bones are highly vascularised and are therefore subject to any systemic effects [306]. Factors that have the potential to disturb bone nutrition and repair may impair the supply of nutrients and oxygen to the overlying cartilage plate [274-276]. Bone death may also occur resulting in a reduced strength of the bony foundation of articular cartilage [274, 275].

Cigarette smoking has been found to be associated with loss of knee cartilage and development of cartilage defects [227-229]. Given the fact that cartilage has no vascular supply the mechanisms by which smoking affects cartilage is unclear. It is possible that any affects smoking has on cartilage are mediated through the underlying bone. While smoking has been shown to be detrimental to bone [334], there have been no studies examining the relationship between smoking and change in knee structures in healthy asymptomatic individuals without knee OA.

The first paper presented within this chapter examines the relationship between smoking and change in tibial cartilage volume and BMLs over 2 years in a cohort of asymptomatic community based individuals. It suggests a possible mechanism by which smoking leads to increased cartilage loss via impairing the ability for BMLs to resolve.

7.1 **Davies-Tuck ML**, Wluka AE, Forbes A, Wang Y, English DR, Giles GG & Cicuttini F. Smoking is Associated with Increased Cartilage Loss and Persistence of Bone Marrow Lesions over 2 Years in Community Based Individuals with No Clinical Knee Osteoarthritis. *Rheumatology* 2009 **48**(10):1227-31.

Dietary fatty acid intake has also been implicated in OA [303, 305]. But once again, the mechanism by which fatty acids affect the knee structure and consequently the risk of knee OA has not been fully elucidated. We have recently shown that higher intakes of fatty acids were associated with an increased prevalence of BMLs in a healthy population without clinical knee OA [291]. However there are no longitudinal studies examining the role of fatty acids on change in BML.

The second paper presented within this chapter examines the association between different types of dietary fatty acids and the incidence of BMLs in healthy, community-based, middle-aged men and women with no clinical knee OA. It suggests that increased fatty acid consumption may increase the risk of developing new bone marrow lesions.

7.2 **Davies-Tuck ML** & Wang Y, Wluka AE, English DR, Giles GG, O'Sullivan R and Cicuttini F. Dietary Fatty Acid Intake affects the risk of developing Bone Marrow Lesions in Healthy Middle-Aged Adults without Knee Osteoarthritis. *Arthritis Research and Therapy* 2009 **11**(3) R63.

The above studies suggest that systemic factors may be associated with OA development via effects on the bone; in addition there is further evidence that OA may have a vascular basis. Hypercholesterolemia and hypertriglyceridemia, both risk factors for cardiovascular disease, have also been related to risk and progression of OA [274, 308]. It is possible that elevated serum lipids make contribute to venous occlusion resulting in small vessel stasis underlying the cartilage plate, joint hypertension, hypercoagulability and/or microemboli which may result in subchondral bone ischemia [274].

The final paper in this chapter aims to further explore the hypothesis that OA may have a vascular basis. It explores the relationship between serum lipids and bone marrow lesions and cartilage over two years in a population of pain free, middle-aged women. We found that serum cholesterol and triglyceride levels were associated with the incidence of BMLs over 2 years. This provides support for the hypothesis that vascular pathology may have a role in the pathogenesis of knee OA. Further work is

warranted to clarify this and whether treatments aimed at reducing serum lipids may have a role reducing the burden of knee OA.

7.3 **Davies-Tuck ML,** Hanna F, Davis SR, Bell R, Davison S, Wluka A, Adams J & Cicuttini FM. Increased Total Cholesterol and Triglycerides are Associated with the Incidence of Bone Marrow Lesions in Asymptomatic Middle-Aged Women. **Arthritis Research and Therapy 2009 11**(6):R181

PART B: Suggested Declaration for Thesis Chapter

Monash University

Declaration for Thesis Chapter 7: Smoking is Associated with Increased Cartilage Loss and Persistence of Bone Marrow Lesions over 2 Years in Community Based Individuals with No Clinical Knee Osteoarthritis

Declaration by candidate

In the case of Chapter 7, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Literature review, measurement of bone marrow lesions, analysis and interpretation of results, manuscript draft preparation	70

The following co-authors contributed to the work. Co-authors who are students at Monash University must also indicate the extent of their contribution in percentage terms:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Anita Wluka	Study design, recruitment of subjects interpretation, data collection, draft revision	
Andrew Forbes	Statistical advice, draft revision	
Yuanyuan Wang	Measurement of cartilage and draft revision	
Dallas English	Subject recruitment and draft revision	
Graham Giles	Subject recruitment and draft revision	
Flavia Cicuttini	Study design, recruitment of subjects, data collection, interpretation, draft revision	

Candidate's Signature		Date

Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
(4) there are no other authors of the publication according to these criteria;
(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
(6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location(s) Department of Epidemiology and Preventive Medicine, Monash University

[Please note that the location(s) must be institutional in nature, and should be indicated here as a department, centre or institute, with specific campus identification where relevant.]

Signature 1

Signature 2

Signature 3

Signature 4

Signature 5

Signature 6

.....

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
(4) there are no other authors of the publication according to these criteria;
(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
(6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location(s) Department of Epidemiology and Preventive Medicine, Monash University

[Please note that the location(s) must be institutional in nature, and should be indicated here as a department, centre or institute, with specific campus identification where relevant.]

Signature 1

Signature 2

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Signature 5

Signature 6

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Smoking is associated with increased cartilage loss and persistence of bone marrow lesions over 2 years in community-based individuals

Miranda L. Davies-Tuck[1], Anita E. Wluka[1,2], Andrew Forbes[1], Yuanyuan Wang[1], Dallas R. English[3,4], Graham G. Giles[4] and Flavia Cicuttini[1]

Objective. To determine whether smoking is related to change in tibial and patella cartilage, and the development or persistence of bone marrow lesions (BMLs) over 2 years in a cohort of middle-aged adults.

Methods. Two hundred and seventy-one adult subjects recruited from the Melbourne Collaborative Cohort Study underwent an MRI of their dominant knee at baseline and ~2 years later. Cartilage volume and BMLs were determined for both time points. At baseline, subjects also completed a questionnaire about current and past cigarette smoking.

Results. Being a 'smoker' (former or current) was associated with increased annual loss of medial but not lateral or patella cartilage volume (medial: difference = 13.4 μl, $P = 0.03$; lateral difference = 4.86 μl, $P = 0.45$, patella difference = −2.57 μl, $P = 0.79$). A relationship between increasing pack-years smoked and increased medial cartilage volume loss was also observed ($P = 0.04$). Amongst people who had a BML at baseline, BMLs present in 'ever smokers' were 11.4 [95% confidence interval (CI) 1.54, 89.9; $P = 0.02$] times more likely to persist over 2 years than those present in 'never smokers'. In addition, the relationship between smoking and increased medial cartilage loss for subjects with a BML present at baseline was partially mediated by the persistence of the BMLs over 2 years.

Conclusion. This study contributes to the evidence of a detrimental effect of smoking on joint cartilage. Furthermore, it provides a possible mechanism that the association smoking shares with increased cartilage loss may be mediated via smoking impairing the ability for BMLs to resolve.

KEY WORDS: Bone marrow lesions, Smoking, Cartilage, Osteoarthritis.

Introduction

The relationship between smoking and the development of knee OA remains conflicting [1–9]. Whereas a number of studies have suggested a protective role of cigarette smoking on risk of OA [2, 3, 10, 11], others have reported no association between smoking and development or progression of knee OA [8, 9]. The null findings of these studies may in part be due to the fact that radiographic OA or knee joint replacement were used as the outcome and therefore provided no information about specific structural changes within the knee [12].

More recently, studies employing MRI to assess change in specific knee structures have found smoking to be associated with increased loss of knee cartilage and development of cartilage defects in cohorts mixed in relation to OA diagnosis and/or symptoms [5–7]. In a cohort largely without radiographic knee OA, smoking and pack-years smoked was associated with increased medial and lateral femoral cartilage loss over ~2 years [5]. In a 30-month follow-up of 159 men with symptomatic knee OA, smokers were found to lose more medial tibiofemoral and patella cartilage and had more severe knee pain than non-smokers [6]. More recently, in a population mixed with relation to OA diagnosis, smoking was found to be associated with increased cartilage loss and cartilage defect development primarily in people with a family history of knee OA >2.5 years [7].

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While loss of articular cartilage is considered the hallmark of OA, changes in the highly vascularized bone, in particular bone marrow lesions (BMLs), have also been implicated in the development of the disease [13–15]. Both cartilage and BMLs can be directly measured from MRI. While the mechanism by which smoking affects cartilage remains unclear, smoking has been shown to be detrimental to bone [16]. Despite this, there are no studies examining the relationship between smoking and change in knee structures in healthy asymptomatic individuals without knee OA. Therefore, the aim of our study was to examine the relationship between smoking and change in knee structures, namely change in cartilage volume and BMLs over 2 years in a cohort of asymptomatic community-based individuals.

Methods

Subjects

The study was conducted within the Melbourne Collaborative Cohort Study (MCCS), which is a prospective cohort study of 41 528 residents of Melbourne, Australia, aged between 27 and 75 years (99.3% were aged 40–69 years) at recruitment which occurred between 1990 and 1994, with the aim of examining the role of lifestyle factors in the risk of cancer and heart disease [17]. Participants were recruited via the electoral rolls (registration to vote is compulsory for adults in Australia), advertisements and community announcements in local media (e.g. television, radio and newspapers). Participants for this current study were recruited from the MCCS. As our intent was to investigate subjects with no significant current or past knee disease, individuals were excluded if they had had any of the following: a clinical diagnosis of knee OA as defined by ACR criteria [18]; knee pain lasting for >24 h in the last 5 years; a previous knee injury requiring non-weight bearing treatment for >24 h or surgery (including arthroscopy); a malignancy; or they were unable to complete the study (e.g. proposed relocation); or they had a history of any form of arthritis diagnosed by a medical practitioner. A further exclusion criterion was a contraindication to MRI including pacemaker, metal sutures, presence of shrapnel or iron filings in the eye or

claustrophobia. We invited subjects who fulfilled our inclusion criteria and attended the first year of round 3 follow-up of the MCCS, which commenced in 2003. We used quota sampling whereby recruitment ceased when our target sample of approximately 300 subjects was achieved. By the end of 2004, 297 eligible subjects were recruited into the current study. The study was approved by The Cancer Council Victoria's Human Research Ethics Committee and the Standing Committee on Ethics in Research Involving Humans of Monash University. All participants gave written informed consent.

Anthropometric data

Study participants completed a questionnaire that included information on their demographics at baseline and at the 2-year follow-up. Weight was measured to the nearest 0.1 kg (shoes, socks and bulky clothing removed) using a single pair of electronic scales. Height was measured to the nearest 0.1 cm (shoes and socks removed) using a stadiometer. From these data, BMI (weight/height^2 kg/m^2) was calculated.

Smoking status

Subjects were classified as having 'ever smoked' if they reported smoking at least seven cigarettes or seven pipes of tobacco per week for at least 1 year as previously described [1]. If subjects did not consume this amount, they were classified as having 'never smoked'. Subjects who had 'ever smoked' were also asked about the average number of cigarettes they smoked a day and about the number of years for which they had smoked. From this the 'pack-year' variable was calculated by averaging the number of cigarettes smoked daily, dividing by 20 (considered one pack) and multiplying by the number of years smoked as previously described [19].

MRI

An MRI of the dominant knee (the knee stepped out from) of each participant was performed between October 2003 and December 2004, and ~2 years later, as described on a 1.5-T whole-body MRI unit (Philips, Medical Systems, Eindhoven, The Netherlands) [20]. The following sequence and parameters were used: fat suppressed, gradient recall acquisition in the steady state, three-dimensional T1-weighted (58 ms/123s/55°, repetition time/echo time/flip angle, slice thickness 1.5 mm, field of view 16 cm and matrix 512 × 196 scans. In addition, a coronal T2-weighted fat-saturated acquisition, repetition time 2500–3000 ms, echo time 40 ms, with a slice thickness of 3.0 mm, a 0.3 interslice gap, 1 excitation, a field of view of 11–12 cm and a matrix of 512 × 512 pixels was also obtained [20].

Cartilage volume measurement

The volumes of the individual cartilage plates (medial and lateral tibial and patella) were measured from the total volume by manually drawing disarticulation contours around the cartilage boundaries on each section on a workstation as described [21]. The coefficients of variation (CVs) for the medial, lateral and patella cartilage volume measures were 3.4, 2.0 and 2.6%, respectively [22, 23]. Annual change in cartilage volume was calculated as (follow-up cartilage volume subtracted from initial cartilage volume) divided by the period of time between MRI scans, as described [22].

BML assessment

BMLs were defined as areas of increased signal intensity adjacent to subcortical bone present in either the medial or lateral, distal femur or proximal tibia assessed of coronal T2-weighted fat-saturated images [24]. Two trained observers, who were blinded to patient characteristics, as well as sequence of images, together assessed the presence of lesions for each subject. The presence or absence of a BML was determined. A lesion was defined as present if it appeared on two or more adjacent slices and encompassed at least one-quarter of the width of the tibial or femoral cartilage being examined from coronal images as previously described [14]. The reproducibility for determination of the BML was assessed using 60 randomly selected knee MRIs (κ-value 0.88, $P < 0.001$). BMLs were described as incident if they were present on the follow-up MRI but not on the baseline MRI. BMLs were described as persistent if they were present on both baseline and follow-up MRI in the same location. Figure 1 shows a BML.

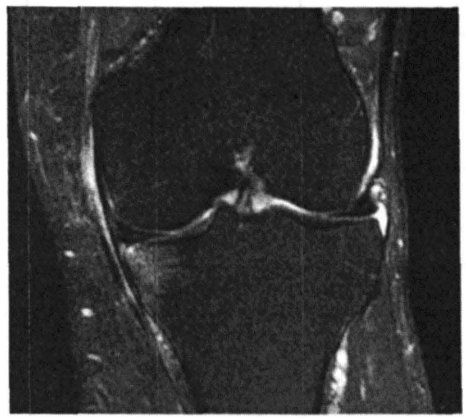

FIG. 1. Coronal T2-weighted fat saturated MRI of a BML.

Statistical analysis

Baseline characteristics, annual change in cartilage volumes and incidence and persistence of BMLs were compared between subjects who had 'never smoked' and those who had 'ever-smoked' ('ex-smokers' and 'current-smokers'), using unpaired *t*-test for continuous variables and chi-squared test for binary variables. Linear regression techniques were used to examine the relationship between smoking and annual change in cartilage volumes if 'ever smoked' and per unit 'pack-year' smoked. Potential confounders including age, gender, BMI, respective baseline tibial plateau area or patella bone volume were included in the multivariate model. Logistic regression was used to determine the odds of having an 'incident' or 'persistent' BML at follow-up if 'ever smoked' and per unit pack-year smoked. Potential confounders of age, gender and BMI were included in the multivariate model. BMLs have been shown to be associated with cartilage loss [13, 25, 26]. Potential mediation by BML persistence of the effect of smoking on knee structure among subjects with a baseline BML was assessed by simultaneous regression equations [27], which yielded indirect (i.e. via persistence of BML) and direct effects of smoking on cartilage loss. A $P < 0.05$ (two-tailed) was regarded as statistically significant. All analyses were performed using the SPSS statistical package (version 15.0.0, SPSS, Cary, NC, USA), apart from the mediation analyses, which used MPlus (version 3.0, Muthen and Muthen, Los Angeles, CA, USA, 2004).

Results

Two hundred and seventy-one (90%) of the 297 participants completed the 2-year follow-up. Reasons for loss to follow-up included death (3), withdrawal for health reasons (4), withdrawal of consent (10), ineligible for follow-up (pacemakers) (4) and

TABLE 1. Characteristics of study participants

	Smokers (former/current), $n=104$	Non-smokers (never), $n=167$	P-value†
Age, years	58.2 ± 5.8	57.6 ± 4.8	0.29
Gender, female, %	64 ± 61	105 ± 63	0.83*
BMI, kg/m^2	25.4 ± 4.1	25.9 ± 4.1	0.38
Baseline cartilage volume			
Medial tibial, μl	1726 ± 504	1688 ± 528	0.56
Lateral tibial, μl	2118 ± 633	2005 ± 637	0.16
Patella, μl	2942 ± 973	2903 ± 923	0.74
Prevalence of BMLs at baseline, n (%)	11 (10)	26 ± 15	0.24*

Values reported as mean ± S.D. except where otherwise stated; †unpaired t-test for difference, except where indicated; *Pearson chi-squared test for dichotomous variable.

TABLE 2. Relationship between smoking and annual change in cartilage volume

	Univariate analysis Regression coefficient (95% CI)	P-value	Multivariate analysis Regression coefficient (95% CI)	P-value
Ever smoked				
Annual change in medial cartilage volume	11.8 (−0.35, 24)	0.057	13.4 (1.32, 25.5)[a]	0.03
Annual change in lateral cartilage volume	5.83 (−6.65, 18.31)	0.36	4.86 (−7.82, 17.5)[a]	0.45
Annual change in patella cartilage volume	−3.75 (−23.2, 15.7)	0.70	−2.57 (−21.89, 16.74)[a]	0.79
Amount of pack-years smoked				
Annual change in medial cartilage volume	0.59 (0.01, 1.18)	0.048	0.63 (0.04, 1.21)[b]	0.04
Annual change in lateral cartilage volume	0.31 (−0.27, 0.90)	0.29	0.29 (−0.31, 0.89)[b]	0.34
Annual change in patella cartilage volume	0.26 (−0.68, 1.21)	0.58	0.27 (−0.68, 1.21)[b]	0.58

[a]Annual change in respective cartilage volume (microlitres) if 'ever smoked' after adjusting for age, gender, BMI and baseline tibial plateau area (or patella bone volume in the case of patella cartilage); [b]annual change in respective cartilage volume (microlitres) per unit pack-year smoked after adjusting for age, gender, BMI and baseline tibial plateau area (or patella bone volume in the case of patella cartilage).

TABLE 3. Relationship between smoking and incidence or persistence of BMLs over 2 years

	Univariate analysis OR (95% CI)	P-value	Multivariate analysis OR (95% CI)	P-value
Ever smoked				
Incident BML	0.98 (0.46, 2.09)	0.96	0.95 (0.43, 2.1)[a]	0.91
Persistent BML	6.14 (1.1, 34.2)	0.04	11.4 (1.54, 89.9)[b]	0.02
Amount of pack-years smoked				
Incident BML	0.98 (0.94, 1.03)	0.47	0.98 (0.94, 1.03)[c]	0.45
Persistent BML	1.13 (0.97, 1.31)	0.12	1.14 (0.97, 1.3)[d]	0.11

[a]OR for developing a BML at follow-up if 'ever smoked' after adjusting for age, gender and BMI; [b]OR for having a BML persist at follow up if 'ever smoked' after adjusting for age, gender and BMI; [c]OR for developing a BML at follow-up per unit pack-year smoked after adjusting for age, gender and BMI; [d]OR for having a BML persist at follow-up per unit pack-year smoked after adjusting for age, gender and BMI. OR: odds ratio.

inability to be contacted (5). The only significant difference between those who completed follow-up and those who were lost to follow-up was that those lost to follow-up were slightly heavier ($P=0.01$). There was no difference in the smoking variables 'ever smoked' ($P=0.12$) and 'pack-years smoked' ($P=0.09$) between those who did and did not complete follow-up.

Of the 271 participants, 104 were classified as 'smokers' (former or current) and 167 as non-smokers (never smoked). There were no significant differences in baseline medial ($P=0.56$), lateral ($P=0.16$) and patella ($P=0.07$) cartilage volumes and also baseline BML prevalence ($P=0.24$) for smokers compared with never smokers (Table 1). At baseline there were 37 prevalent BMLs: over the 2-year study period 20 of these BMLs persisted whereas the other 17 completely resolved. In addition, among the 234 subjects who did not have a BML present in their knee, 33 developed a BML over the 2-year study period.

Compared with 'non-smokers', being a 'smoker' (former or current) was associated with increased annual loss of medial tibial cartilage [regression coefficient $\beta=13.4\,\mu$l, 95% confidence interval (CI) 1.32, 25.5; $P=0.03$], but not lateral or patella cartilage volume after adjustment for potential confounders. Further, for every unit of 'pack-years' smoked there was an associated increased annual medial tibial cartilage loss (regression coefficient $\beta=0.63\,\mu$l, 95% CI 0.04, 1.21; $P=0.04$) after adjusting for potential confounders. No association between 'pack-years' smoked and annual change in lateral tibial or patella cartilage volume was observed (Table 2). Similar findings were observed when current smokers were compared with non-smokers, but the results were not significant as there were only 18 subjects in this group (data not shown).

Whereas no association between smoking and the development of an incident BML over 2 years was observed, in people who had a BML at baseline, those who were 'ever smokers' were 11.4 (95% CI 1.54, 89.9) times more likely to persist at follow-up after adjusting for potential confounders ($P=0.02$). No association between the persistence of BMLs and 'pack-years' smoked was observed (Table 3).

In order to assess whether the significant relationship between smoking and increased medial cartilage loss was mediated by the persistence of a BML over 2 years, the association between persistent BMLs, medial cartilage loss and smoking was explored. To show mediation [27], not only did smoking need to be related to both BML persistence and annual change in cartilage volume, BML persistence also needed to be associated with annual change in cartilage volume. Where a BML persisted over 2 years, medial tibial cartilage loss was significantly increased by 31.8 μl (95% CI 4.8, 58.9, $P=0.02$) after adjusting for age, gender, BMI and baseline medial tibial bone. Therefore, having a persistent BML was included in the multivariate model examining the relationship between smoking and annual change in medial cartilage volume.

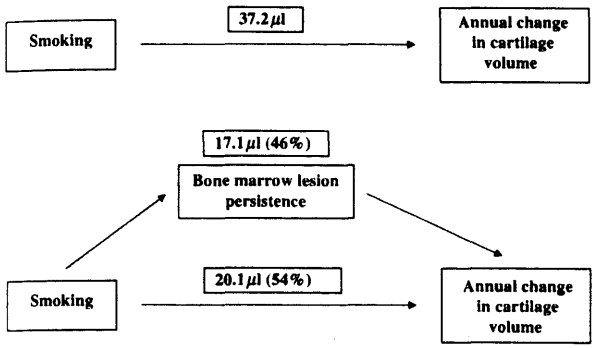

FIG. 2. Path analysis displaying the contribution of mediation by BML persistence in the relationship between smoking and annual change in medial cartilage volume. Forty-six percent of the difference in annual change in cartilage volume between smokers and never smokers was due to an indirect pathway through BML persistence. The remaining 54% was via a direct relationship (or via other unknown pathways) between smoking and cartilage change.

Ideally, if complete mediation existed, smoking should no longer be associated with annual cartilage volume change when BML persistence was included in the regression model. When 'persistent' BMLs were included within the model the association between 'ever smoked' and annual change in medial cartilage volume no longer remained [regression coefficient for if 'ever smoked' 26.75 (−6.41, 59.9), $P=0.11$)].

Pathway analysis was then performed to determine the contribution of mediation of BML persistence in the relationship between smoking and annual change in medial cartilage volume for people with a BML present in their knee at baseline. Here, it was found that the total effect of smoking on change in cartilage in people who had a BML present at baseline was 37.2 μl (95% CI 6.21, 68.18, $P=0.02$) (after adjusting for confounders). Thus, 46% (17.1 μl) of the difference in annual change in cartilage volume between smokers and never smokers was due to an indirect pathway through BML persistence. The remaining 54% (20.1 μl) was via a direct relationship (or via other unknown pathways) between smoking and cartilage change (Fig. 2)

Discussion

In this cohort study of asymptomatic, community-based adults, a history of smoking (current and past) was associated with increased medial tibial, but not lateral tibial or patella cartilage loss over 2 years. In addition, there was a dose–response relationship between 'pack-years' smoked and increased medial tibial cartilage loss. For individuals who had a BML at baseline, smoking was also associated with the persistence of the BML over 2 years. The persistence of the BML was found to partially mediate the relationship between smoking and cartilage loss.

This is the first longitudinal study to examine the relationship between smoking and change in cartilage volume in an asymptomatic, community-based population. Our finding that smoking was associated with increased medial tibial cartilage loss is consistent with other studies that have examined the relationship between smoking and cartilage using MRI [5–7]. Smoking was found to be associated with increased medial and lateral femoral cartilage loss over ~2 years in a cohort largely without knee radiographic knee OA [5]. Similarly, smokers were found to lose more medial tibiofemoral and patella cartilage and had more severe knee pain than non-smokers over a 30-month follow-up of 159 men with symptomatic knee OA [6]. More recently, in a population mixed with relation to OA diagnosis, smoking was found to be associated with increased cartilage loss and cartilage defect development primarily for people with a family history of knee OA over 2.5 years [7]. Taken together with the findings of our study, these data support a negative effect of smoking on knee cartilage.

Although we found no association between smoking and the development of incident BMLs over 2 years, smoking was associated with the persistence of BMLs present at baseline. To our knowledge, no previous study has examined this. The aetiology of BMLs in healthy asymptomatic subjects is unclear. On histopathologic examination, BMLs demonstrate features that include oedema, osteonecrosis, abnormal bone formation with excessive fibrosis and extensive bony remodelling [28]. Cigarette smoking has been shown to impair bone healing [16]. Nicotine also diminishes osteoblast function [29] and increases carbon monoxide levels in arterial blood contributing to tissue hypoxia [30], which consequently induces osteoclast activity and bone resorption [31]. A study of smokers with tibial shaft fractures found that smokers took longer to heal than non-smokers [32]. Similarly, it might be that once a BML has developed smoking reduces the capacity for the BML to resolve.

In this asymptomatic population with no clinical OA, smoking was associated with an increased rate of loss of articular cartilage, a hallmark of OA and persistence of BMLs. BMLs have been associated with structural changes in the knee, including joint space narrowing [33], increased prevalence and severity of cartilage defects [34–36] and loss of cartilage [13, 25, 26]. We found that the association between smoking and increased medial cartilage loss was mediated by the persistence of a BML over 2 years. BMLs may represent one step in the continuum from a healthy to a diseased joint. In OA/symptomatic populations, <10% of BMLs resolve [26]. In contrast, in this asymptomatic population approximately half of the BMLs present at baseline resolved and persistence of BMLs was associated with smoking. Taken together, this suggests that the detrimental effect of cigarette smoking on medial tibial cartilage maybe be partially mediated via the effect of cigarette smoke reducing the capacity of the BML to resolve.

This study has a number of potential limitations. First, it examined a healthy, community-based population selected on the criteria of no knee pain or injury and, therefore, the results are not generalizable to symptomatic populations or people who have injured their knees. However, the findings of our study can be generalized to populations that may be targeted for primary prevention. In addition, as participants were healthy volunteers, there is the possibility that they also engaged in a healthier lifestyle and may therefore be less likely to smoke and more likely to exercise. If this is the case, however, this would under- rather then overestimate associations observed. Furthermore, ~40% of our population were current or past smokers, and that combined with the mean BMI being within the overweight the effect of this potential bias is likely to be limited. Secondly, we did not obtain radiographs of the knees, so subjects may have had asymptomatic, radiographic OA. However, we used the ACR clinical criteria of OA to determine status of knees and individuals with significant knee injury in the past, pain at baseline, knee surgery or medical diagnosis of any other type of arthritis were excluded [37]. Due to the small number of persistent BMLs, we were unable to examine whether smoking is associated with increases or decreases in their size, larger studies would be required to assess this. We also did not assess knee alignment, which has been shown to be associated with the presence of BMLs [38]; but it is possible that the relationship between alignment and BMLs is mediated through increased loading on the joint. Therefore, our adjustment for BMI should reduce this limitation. Our classification of 'ever smoked' may have introduced some misclassification bias; however, this would have underestimated the associations between smoking and cartilage and BMLs rather than overestimated them. Finally, although femoral cartilage was not examined in this study, change in femoral cartilage volume correlates with tibial cartilage volume both cross-sectionally [39] and longitudinally [40].

In this study of asymptomatic middle-aged adults with no clinical knee OA, cigarette smoking was associated with greater medial tibial cartilage loss and the persistence of BMLs over 2 years. This study provides a possible mechanism for the previously observed negative association of smoking with structural knee changes associated with development of knee OA and suggests that the association smoking shares with increased cartilage loss may be partially mediated via smoking impairing the ability for BMLs to resolve.

Rheumatology key messages

- Cigarette smoking was associated with greater medial tibial cartilage loss and the persistence of BMLs.
- The association smoking shares with cartilage loss may be mediated via its effect on BMLs.

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Disclosure statement: The authors have declared no conflicts of interest.

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PART B: Suggested Declaration for Thesis Chapter

Monash University

Declaration for Thesis Chapter 7: Dietary Fatty Acid Intake affects the risk of developing Bone Marrow Lesions in Healthy Middle-Aged Adults without Knee Osteoarthritis

Declaration by candidate

In the case of Chapter 7, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Literature review, measurement of bone marrow lesions, analysis and interpretation of results, manuscript draft preparation	65

The following co-authors contributed to the work. Co-authors who are students at Monash University must also indicate the extent of their contribution in percentage terms:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Yuanyuan Wang	Joint 1st author. Literature review, data collection, measurement of cartilage and draft revision	
Anita Wluka	Recruitment of subjects, interpretation, draft revision	
Dallas English	Subject recruitment and draft revision	
Graham Giles	Subject recruitment and draft revision	
Richard O'Sullivan	Draft revision	
Flavia Cicuttini	Study design, recruitment of subjects, data collection, interpretation, draft revision	

Candidate's Signature		Date

Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
(4) there are no other authors of the publication according to these criteria;
(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
(6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location(s) Department of Epidemiology and Preventive Medicine, Monash University

[Please note that the location(s) must be institutional in nature, and should be indicated here as a department, centre or institute, with specific campus identification where relevant.]

Signature 1

Signature 2

Signature 3

Signature 4

Signature 5

Signature 6

.....

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.
(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
(4) there are no other authors of the publication according to these criteria;
(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
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Location(s) Department of Epidemiology and Preventive Medicine, Monash University

[Please note that the location(s) must be institutional in nature, and should be indicated here as a department, centre or institute, with specific campus identification where relevant.]

Signature 1

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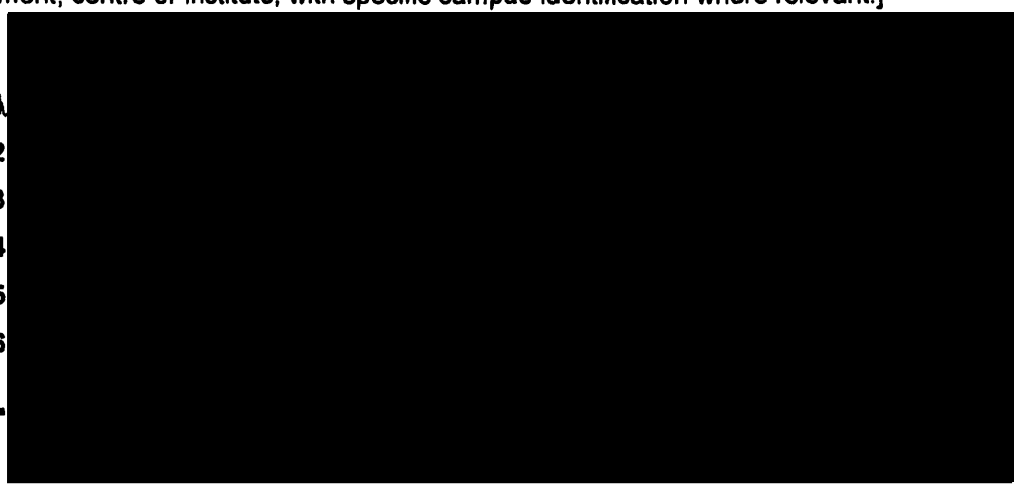

Research article

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Dietary fatty acid intake affects the risk of developing bone marrow lesions in healthy middle-aged adults without clinical knee osteoarthritis: a prospective cohort study

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Abstract

Introduction Fatty acids have been implicated in osteoarthritis (OA), yet the mechanism by which fatty acids affect knee structure and consequently the risk of knee OA has not been fully elucidated. Higher intakes of fatty acids have been shown to be associated with the risk of bone marrow lesions (BMLs) in a healthy population. The aim of this study was to examine the association between fatty acid consumption and the incidence of BMLs in healthy middle-aged adults without clinical knee OA.

Methods Two hundred ninety-seven middle-aged adults without clinical knee OA underwent magnetic resonance imaging (MRI) of their dominant knee at baseline. BMLs were assessed. Of the 251 participants with no BMLs in their knee at baseline, 230 underwent MRI of the same knee approximately 2 years later. Intakes of fatty acids were estimated from a food frequency questionnaire.

Results Increased consumption of saturated fatty acids was associated with an increased incidence of BMLs over 2 years after adjusting for energy intake, age, gender, and body mass index (odds ratio of 2.56 for each standard deviation increase in dietary intake, 95% confidence interval 1.03 to 6.37, $P = 0.04$). Intake of monounsaturated or polyunsaturated fatty acids was not significantly associated with the incidence of BMLs.

Conclusions Increased fatty acid consumption may increase the risk of developing BMLs. As subchondral bone is important in maintaining joint integrity and the development of OA, this study suggests that dietary modification of fatty acid intake may be one strategy in the prevention of knee OA which warrants further investigation.

Introduction

Nutritional factors have been shown to be important in the maintenance of bone and joint health [1]. In particular, fatty acids have been implicated in osteoarthritis (OA) [2,3]. Elevated levels of fat and n-6 polyunsaturated fatty acids have been found in OA bone [2], whereas n-3 polyunsaturated fatty acids have been shown to alleviate progression of OA through an effect on the metabolism of articular cartilage [3]. Although dietary supplementation with polyunsaturated fatty acids has been shown to decrease bone turnover and increase bone mineral density [4], the finding that a higher ratio of n-6 to n-3 polyunsaturated fatty acids is associated with lower bone mineral density at the hip [5] suggests the important role of rela-

BMI: body mass index; BML: bone marrow lesion; CI: confidence interval; MCCS: Melbourne Collaborative Cohort Study; MRI: magnetic resonance imaging; OA: osteoarthritis; SD: standard deviation.

tive amounts of these polyunsaturated fatty acids in preserving skeletal integrity in older age.

However, the mechanism by which polyunsaturated fatty acids affect the knee structure and consequently the risk of knee OA has not been fully elucidated. We have recently shown that higher intakes of monounsaturated, total, and n-6 polyunsaturated fatty acids were associated with an increased prevalence of bone marrow lesions (BMLs) in a healthy population without clinical knee OA [6]. BMLs have been associated with structural changes of disease severity, including increased cartilage defects, tibial plateau area, loss of cartilage, and joint space narrowing, suggesting that they play a role in the pathogenesis of OA [7-9]. However, there are no longitudinal studies examining the role of fatty acids on incident BMLs in either healthy or OA populations. Therefore, the aim of this study was to examine the association between intakes of different types of fatty acids and the incidence of BMLs in healthy, community-based, middle-aged men and women with no clinical knee OA.

Materials and methods

Subjects

This study was conducted within the Melbourne Collaborative Cohort Study (MCCS), a prospective cohort study of 41,528 Melbourne, Australia residents who were 40 to 69 years old at recruitment (1990 to 1994) [10]. Participants for the current study were recruited from within the MCCS between 2003 and 2004 as previously described [6]. Briefly, participants were eligible if they were between 50 and 79 years old without any of the following exclusion criteria: a clinical diagnosis of knee OA as defined by American College of Rheumatology criteria [11], knee pain lasting for more than 24 hours in the last 5 years, a previous knee injury requiring non-weight-bearing treatment for more than 24 hours or surgery (including arthroscopy), or a history of any form of arthritis diagnosed by a medical practitioner. A further exclusion criterion was a contraindication to magnetic resonance imaging (MRI), including pacemaker, metal sutures, presence of shrapnel or iron filings in the eye, or claustrophobia. The study was approved by The Cancer Council Victoria's Human Research Ethics Committee and the Standing Committee on Ethics in Research Involving Humans of Monash University. All participants gave written informed consent.

Anthropometric and dietary data

Height was measured using a stadiometer with shoes removed. Weight was measured using electronic scales with bulky clothing removed. Body mass index (BMI) (weight/height^2, kg/m^2) was calculated. At MCCS baseline, questionnaires covered demographic data and diet (via a 121-item food frequency questionnaire developed from a study of weighed food records [12]). Fatty acid intakes were calculated from the food frequency questionnaire using Australian food composition data and were adjusted for energy intake [13].

Magnetic resonance imaging and the measurement of bone marrow lesions

Each subject had an MRI performed on the dominant knee, determined from kicking preference [14], at baseline and approximately 2 years later. Knees were imaged on a 1.5-T whole-body magnetic resonance unit (Philips Medical Systems, Eindhoven, The Netherlands) using a commercial transmit-receive extremity coil, with coronal T_2-weighted fat-saturated acquisition as previously described [9]. BMLs were defined as areas of increased signal intensity adjacent to subcortical bone present in either the medial or lateral, distal femur or proximal tibia [9]. Two trained observers, blinded to patient characteristics and sequence of images, together assessed the presence of lesions for each subject. The baseline and follow-up images were assessed unpaired. A lesion was defined as present if it appeared on two or more adjacent slices and encompassed at least one quarter of the width of the tibial or femoral cartilage being examined from coronal images, equivalent to a 'large BML' as described by Felson and colleagues [9]. The reproducibility for determination of BMLs was assessed using 60 randomly selected knee MRIs (κ value 0.88, $P < 0.001$).

Statistical analyses

The descriptive statistics of the characteristics of study participants were tabulated. Participants with self-reported total energy intakes in the top or bottom 1% of the gender-specific distributions were excluded. A BML was defined as incident if it was present at follow-up in the knees without BMLs at baseline. Logistic regression models were constructed to explore the relationship between fatty acid intakes and incident BMLs after adjusting for potential confounders of age, gender, BMI, and energy intake. Intake of fatty acids was standardised so that the coefficients represent the effect of an increment of one standard deviation (SD) in intake. P values of less than 0.05 were considered to be statistically significant. All analyses were performed using the SPSS statistical package (standard version 15.0.0; SPSS Inc., Cary, NC, USA).

Results

Two hundred ninety-seven subjects entered the study, and four subjects were excluded due to having energy intakes in the top or bottom 1% of the gender-specific distributions. Of the 251 participants who did not have a BML at baseline, 230 (92%) completed the 2-year follow-up. Participants lost to follow-up had a higher BMI ($P = 0.04$) compared with those who completed follow-up. There were no significant differences in consumption of saturated ($P = 0.56$), monounsaturated ($P = 0.59$), or polyunsaturated ($P = 0.75$) fatty acids between the two groups. Thirty-two subjects developed BMLs at follow-up. Participants who developed BMLs had a higher BMI (mean [SD] 27.9 [5.3] versus 25.4 [3.8] kg/m^2, $P = 0.02$) and higher

energy intake-adjusted saturated fatty acid consumption (mean [standard error] 35.7 [1.2] versus 33.0 [0.5] g/day, $P = 0.03$) when compared with those who did not. There was no significant difference in terms of the energy intake-adjusted consumption of monounsaturated and polyunsaturated fatty acids (Table 1).

Although there was no significant association between fatty acid consumption and the incidence of BMLs over 2 years in univariate analysis, higher consumption of saturated fatty acids was significantly associated with an increased risk of developing BMLs after adjusting for energy intake (Table 2, model 1). For each SD increase in dietary intake of saturated fatty acids, the risk of developing BMLs over 2 years increased 2.62-fold (95% confidence interval [CI] 1.11 to 6.17). This relationship persisted after further adjusting for age, gender, and BMI (odds ratio 2.56, 95% CI 1.03 to 6.37) (Table 2, model 2). No significant association between consumption of monounsaturated or polyunsaturated fatty acids or n-6/n-3 ratio and incident BMLs was found in multivariate analyses (Table 2).

From MCCS baseline when dietary fatty acid intake data were collected during 1990 to 1994 to the inception of current study when baseline MRI was performed in 2003 to 2004, the weight of participants increased by a mean of 2.1 kg (SD 5.2 kg). After adding weight gain to model 2, consumption of saturated fatty acids persisted to be positively associated with incident BMLs (odds ratio 2.54, 95% CI 1.01 to 6.39).

There was no evidence that BMI modified the association between energy intake-adjusted dietary saturated fatty acid consumption and incident BMLs when an interaction term for BMI category × saturated fatty acid intake was included in the logistic model with adjustment for energy intake. The P value was 0.64 when BMI was categorised as less than 25 kg/m^2, 25 to 30 kg/m^2, and greater than or equal to 30 kg/m^2.

Discussion

In a population of healthy middle-aged adults with no clinical knee OA, we found that higher intake of saturated, but not monounsaturated or polyunsaturated, fatty acids or that the n-6/n-3 ratio was associated with an increased likelihood of developing BMLs over 2 years. This is the first longitudinal study presenting a relationship between dietary fatty acid intake and the incidence of BMLs. We have previously shown in a cross-sectional study that increased dietary intake of monounsaturated and n-6, but not n-3, polyunsaturated fatty acids were associated with an increased risk of having BMLs in a healthy population without clinical knee OA [6]. When this population was followed up for 2 years, we found an association between higher saturated fatty acid intake and increased likelihood of developing BMLs over 2 years. Although the mechanism for the discrepancy in terms of the type of fatty acid consumption observed between the previous cross-sectional study and the current prospective cohort study is unclear, the adverse effect of saturated fatty acids on the incidence of BMLs may be attributed to a vascular effect. Saturated fatty acid intake has been associated with atherosclerosis and cardiovascular disease [15]. There are no previous studies identifying a relationship between saturated fatty acid intake and the risk of OA. Recently, it has been suggested that atheromatous vascular disease may be important in the progression of OA [16] and that subchondral ischaemia may be a mechanism by which vascular pathology plays a role in the initiation and/or progression of OA [17]. The findings of this study therefore suggest that vascular disease in subchondral bone may play a role in the pathogenesis of OA via BMLs.

Table 1

Characteristics of study participants with no bone marrow lesions at baseline

	Incident BMLs (n = 32)	Without incident BMLs (n = 198)	P value[a]
Age, years	57.6 (5.8)	57.7 (5.0)	0.91
Number of females (percentage of females)	23 (72%)	120 (61%)	0.22[b]
Body mass index, kg/m^2	27.9 (5.3)	25.4 (3.8)	0.02
Energy intake, kJ/d	8,822 (3,019)	9,293 (3,063)	0.42
Saturated fatty acid, g/day	35.7 (1.2)	33.0 (0.5)	0.03[c]
Monounsaturated fatty acids, g/day	29.3 (0.9)	27.9 (0.4)	0.14[c]
Polyunsaturated fatty acids, g/day	12.7 (0.7)	12.5 (0.3)	0.76[c]
n-3 polyunsaturated fatty acids, g/day	1.2 (0.05)	1.2 (0.02)	0.60[c]
n-6 polyunsaturated fatty acids, g/day	11.3 (0.6)	11.4 (0.3)	0.92[c]
n-6/n-3 ratio	9.6 (0.5)	9.7 (0.2)	0.82[c]

Data are presented as mean (standard deviation) unless otherwise stated. [a]P value for comparisons between two groups using independent samples t test, [b]chi-square test, or [c]one-way analysis of covariance after adjusting for energy intake. BMLs, bone marrow lesions.

Table 2

Relationship between fatty acid intake and incidence of bone marrow lesions

	Univariate analysis, OR (95% CI)	*P* value	Model 1 Multivariate analysis, OR (95% CI)[a]	*P* value	Model 2 Multivariate analysis, OR (95% CI)[b]	*P* value
Saturated fatty acids	1.08 (0.72–1.60)	0.73	2.62 (1.11–6.17)	0.03	2.56 (1.03–6.37)	0.04
Monounsaturated fatty acids	1.01 (0.66–1.52)	0.98	2.10 (0.81–5.47)	0.13	1.99 (0.75–5.31)	0.17
Polyunsaturated fatty acids	0.94 (0.62–1.42)	0.77	1.10 (0.64–1.90)	0.74	1.10 (0.62–1.96)	0.74
n-6 polyunsaturated fatty acids	0.88 (0.57–1.35)	0.55	0.98 (0.56–1.70)	0.93	0.98 (0.55–1.76)	0.96
n-3 polyunsaturated fatty acids	0.81 (0.53–1.25)	0.34	0.85 (0.46–1.56)	0.60	0.85 (0.45–1.61)	0.62
n-6/n-3 ratio	0.94 (0.63–1.38)	0.74	0.96 (0.65–1.41)	0.82	0.93 (0.61–1.42)	0.74

[a]Model 1: odds ratio for development of tibiofemoral bone marrow lesions for each increase of 1 standard deviation in the respective fatty acid intake after adjusting for energy intake. [b]Model 2: odds ratio for development of tibiofemoral bone marrow lesions for each increase of 1 standard deviation in the respective fatty acid intake after adjusting for energy intake, age, gender, and body mass index. CI, confidence interval; OR, odds ratio.

There is mounting evidence that BMLs play a role in the pathogenesis of OA [7-9]. It has been demonstrated that BMLs are associated with the presence of cartilage defects in healthy asymptomatic populations with no history of significant knee pain or injury and that risk factors for OA such as age, height, and BMI also affect the prevalence of BMLs [18,19]. Moreover, the presence of BMLs predicts the progression of cartilage defects and loss of cartilage volume over 2 years in longitudinal studies [20,21]. These findings suggest that BMLs may be associated with an increased risk of knee OA. This study demonstrates an increased incidence of BMLs associated with increased saturated fatty acid intake in a healthy population and suggests that modifying diet may be one such way to reduce the development and subsequent burden of OA.

This study has a number of potential limitations. First, this study examined a healthy, community-based population selected on the criterion of having no knee pain or injury and therefore the results may not be generalisable to symptomatic populations or people who have injured their knees. However, the findings of our study can be generalised to populations that would be targeted by primary prevention strategies. Second, whilst the dietary intake of fatty acids was measured in a valid fashion [22], this was based on a single measure of nutrient intakes 10 years earlier. Although significant underreporting of fat intake is likely [23], absolute intake of dietary fat tends to remain stable [24,25]. While nutritional data collected 10 years earlier may have resulted in some misclassification of exposure, such misclassification is likely to have been non-differential in relation to knee structure since only subjects with no history of knee symptoms or injury were included, thereby tending to underestimate the strength of any observed associations. In the current study, we did not measure knee alignment, which has been shown to be associated with BMLs [9].

Conclusions

The findings of this study suggest that increased fatty acid consumption may increase the risk of developing BMLs in a healthy population without clinical knee OA. As subchondral bone is important in maintaining joint integrity and the development of OA, this study suggests that dietary modification of fatty acid intake may be one strategy in the prevention of knee OA which warrants further investigation.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

YW participated in the design of the study, performed the statistical analysis and the interpretation of data, and drafted the manuscript. MLD-T performed the measurement of bone marrow lesions, participated in the statistical analysis and the interpretation of data, and drafted the manuscript. AEW participated in the interpretation of data and reviewed the manuscript. AF helped in the statistical analysis and reviewed the manuscript. DRE and GGG participated in the design of the study and the acquisition of data and reviewed the manuscript. RO provided technical support and reviewed the manuscript. FMC participated in the design of the study, helped in the interpretation of data, and reviewed the manuscript. All authors read and approved the final manuscript.

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PART B: Suggested Declaration for Thesis Chapter

Monash University

Declaration for Thesis Chapter 7: Increased Total Cholesterol and Triglycerides are Associated with the Incidence of Bone Marrow Lesions in Asymptomatic Middle-Aged Women

Declaration by candidate

In the case of Chapter 7, the nature and extent of my contribution to the work was the following:

Nature of contribution	Extent of contribution (%)
Literature review, measurement of bone marrow lesions, analysis and interpretation of results, manuscript draft preparation	70

The following co-authors contributed to the work. Co-authors who are students at Monash University must also indicate the extent of their contribution in percentage terms:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Fahad Hanna	Measurement of cartilage and draft revision	
Sue Davis	Study design, subject recruitment, interpretation and draft revision	
Anita Wluka	Interpretation, draft revision	
Robin Bell	Study design, subject recruitment, interpretation and draft revision	
Jenny Adams	Subject recruitment	
Flavia Cicuttini	Study design, data collection, interpretation, draft revision	

Candidate's Signature		Date

Declaration by co-authors

The undersigned hereby certify that:

(1) the above declaration correctly reflects the nature and extent of the candidate's contribution to this work, and the nature of the contribution of each of the co-authors.

(2) they meet the criteria for authorship in that they have participated in the conception, execution, or interpretation, of at least that part of the publication in their field of expertise;
(3) they take public responsibility for their part of the publication, except for the responsible author who accepts overall responsibility for the publication;
(4) there are no other authors of the publication according to these criteria;
(5) potential conflicts of interest have been disclosed to (a) granting bodies, (b) the editor or publisher of journals or other publications, and (c) the head of the responsible academic unit; and
(6) the original data are stored at the following location(s) and will be held for at least five years from the date indicated below:

Location(s) Department of Epidemiology and Preventive Medicine, Monash University

[Please note that the location(s) must be institutional in nature, and should be indicated here as a department, centre or institute, with specific campus identification where relevant.]

Signature 1

Signature 2

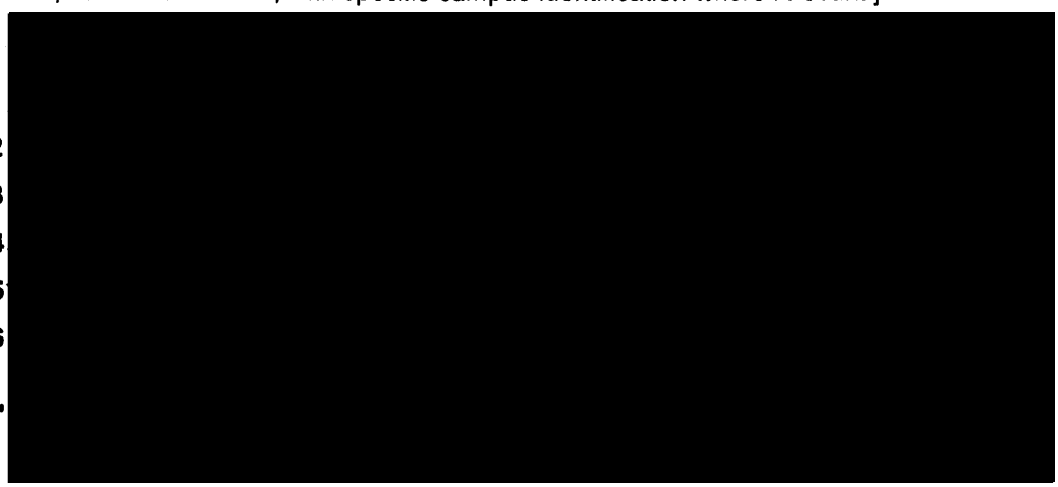
Signature 3

Signature 4

Signature 5

Signature 6

.....



Research article

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Total cholesterol and triglycerides are associated with the development of new bone marrow lesions in asymptomatic middle-aged women - a prospective cohort study

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Abstract

Introduction Given the emerging evidence that osteoarthritis (OA) may have a vascular basis, the aim of this study was to determine whether serum lipids were associated with change in knee cartilage, presence of bone marrow lesions (BMLs) at baseline and the development of new BMLs over a 2-year period in a population of pain-free women in mid-life.

Methods One hundred forty-eight women 40 to 67 years old underwent magnetic resonance imaging (MRI) of their dominant knee at baseline and 2.2 (standard deviation 0.12) years later. Cartilage volume and BMLs were determined for both time points. Serum lipids were measured from a single-morning fasting blood test approximately 1.5 years prior to the MRI.

Results The incidence of BML at follow-up was associated with higher levels of total cholesterol (odds ratio [OR] 1.84, 95% confidence interval [CI] 1.01, 3.36; $P = 0.048$) and triglycerides (OR 8.4, 95% CI 1.63, 43.43; $P = 0.01$), but not high-density lipoprotein (HDL) ($P = 0.93$), low-density lipoprotein (LDL) ($P = 0.20$) or total cholesterol/HDL ratio ($P = 0.17$). No association between total cholesterol, triglycerides, HDL, LDL or total cholesterol/HDL ratio and presence of BMLs at baseline or annual change in total tibial cartilage volume was observed.

Conclusions In this study of asymptomatic middle-aged women with no clinical knee OA, serum cholesterol and triglyceride levels were associated with the incidence of BMLs over 2 years. This provides support for the hypothesis that vascular pathology may have a role in the pathogenesis of knee OA. Further work is warranted to clarify this and whether treatments aimed at reducing serum lipids may have a role in reducing the burden of knee OA.

Introduction

The prevalence of vascular disease and cardiovascular risk factors is high amongst people with osteoarthritis (OA) [1,2]. Emerging evidence suggests that these conditions may share risk factors [1-5]. Hypercholesterolemia and hypertriglyceridemia, both risk factors for cardiovascular disease, have been related to risk of OA and the progression of OA in epidemiologic studies [1-3].

There are a number of mechanisms by which vascular pathology may contribute to the development of OA. The ends of bones are particularly susceptible to vascular insult [6]. Venous occlusion resulting in small-vessel stasis underlying the cartilage plate, joint hypertension, hypercoagulability and/or microemboli may all result in subchondral bone ischemia [3]. The resulting disturbances to subchondral bone nutrition and repair may impair the supply of nutrients and oxygen to the overlying cartilage plate [3,7,8]. Bone ischemia may also result

BMI: body mass index; BML: bone marrow lesion; CI: confidence interval; CV: coefficient of variation; HDL: high-density lipoprotein; HDL-C: high-density lipoprotein cholesterol; LDL: low-density lipoprotein; LDL-C: low-density lipoprotein cholesterol; ln: natural logarithm; MRI: magnetic resonance imaging; OA: osteoarthritis; OR: odds ratio; SD: standard deviation.

in osteocyte death, leading to bone resorption, which may reduce the strength of the bony foundation of articular cartilage [3,8].

With the advent of magnetic resonance imaging (MRI), it is now possible to directly visualise joint structures, including cartilage and bone, in healthy subjects prior to the onset of OA. While cartilage loss is considered the hallmark of OA and is associated with symptoms [9] and risk of joint replacement [10], there is increasing evidence for a significant role of bone in the pathogenesis of knee OA and it has been suggested that bone changes may predate cartilage changes [11].

Bone marrow lesions (BMLs) are associated with knee pain [12-17] and structural changes in the knee in subjects with or without pain or radiographic OA or both. These include increased joint space narrowing [18], loss of cartilage [19-21] and cartilage defects [13,22,23]. BMLs are common in those with OA, are predominantly associated with malalignment and, once present, are unlikely to resolve [24-26]. In contrast, in asymptomatic populations [20,23], their presence is also associated with systemic factors such as dietary lipids [27] and they are more likely to resolve [28]. This is perhaps not surprising given that the histology of BML is heterogeneous and includes osteonecrosis, oedema, trabecular abnormalities and bony remodeling [29] and more recently evidence of ischemia or reperfusion injury or both [8,30]. Given the emerging evidence that OA may have a vascular basis, the aim of this study was to explore the relationship between serum lipids and (a) baseline prevalence of BMLs and (b) annual change in knee cartilage and incidence of BMLs over a 2-year period in a population of pain-free middle-aged women.

Materials and methods

One hundred seventy-six women, 40 to 67 years old, were recruited from an existing cross-sectional study examining knee structure in women [22]. These women were initially recruited from a database established from the electoral roll in Victoria, Australia, between April 2002 and August 2003 [31]. Women were excluded if they had OA as defined by the American College of Rheumatology clinical criteria [32], current or past knee disease, a history in the past 5 years of knee pain lasting for more than 24 hours, a previous knee injury requiring non-weight-bearing treatment for more than 24 hours or surgery (including arthroscopy) or a history of any arthritis diagnosed by a medical practitioner or contraindication to MRI. The study was approved by the Alfred Hospital Human Research Ethics Committee, and all participants gave written informed consent.

Anthropometric data and smoking status

The height and weight of each participant were measured at the time of the original study (2003 to 2005). Body mass index (BMI) was calculated from these data as weight (in kilograms) divided by height squared (in metres squared). Smoking status was determined by questionnaire.

Measurement of blood lipids

Each participant took part in a single-morning fasting blood test at the time of the original study (2002 to 2003) approximately 1.53 years (standard deviation [SD] 0.24 years) prior to their first knee MRI. Fasting bloods drawn at the time of recruitment were stored at -80°C until assayed. Total cholesterol was determined by the CHOD-PAP (cholesterol oxidase phenol 4-aminoantipyrine peroxidase) method and triglycerides by the GPO-PAP (glycerol phosphate oxidase-p-aminophenazone) method using a Hitachi 747 analyser (Boehringer Mannheim Systems, now part of Roche Diagnostics, Basel, Switzerland). High-density lipoprotein cholesterol (HDL-C) was measured by an enzymatic colorimetric test on a Hitachi 747 analyser. The assay range is 0.1 to 20 mg/L with intra-assay coefficients of variation (CVs) of 1.34% at 0.55 mg/L and 0.28% at 12.36 mg/L, interassay CVs of 5.7% at 0.52 mg/L and 2.5% at 10.98 mg/L and a detection limit of 0.03 mg/L [33]. Low-density lipoprotein cholesterol (LDL-C) was calculated according to a method previously described [34].

Magnetic resonance imaging and the measurement of cartilage volume and bone marrow lesion

An MRI of each woman's dominant knee (defined as the lower limb from which the subject stepped off from when initiating gait) was performed between October 2003 and August 2004 and approximately 2 years later [22]. Knees were imaged in the sagittal plane on a 1.5-T whole-body magnetic resonance unit (Philips Medical Systems, Eindhoven, The Netherlands) using a commercial transmit-receive extremity coil. The following sequence and parameters were used: fat-saturated, gradient echo, three-dimensional, T1-weighted (8 ms/12 ms/55 degrees, repetition time/echo time/flip angle, slice thickness of 1.5 mm, field of view of 16 cm and matrix of 513 × 196 pixels). In addition, a coronal, T2-weighted, fat-saturated acquisition (repetition time of 2,200 ms, echo time of 20/80 ms, slice thickness of 3 mm, 0.3 interslice gap, one excitation, field of view of 11 to 12 cm and matrix of 256 × 128 pixels) was obtained.

Assessment of bone marrow lesions

BMLs were defined as areas of increased signal intensity adjacent to subcortical bone present in either the medial or lateral side of the distal femur or proximal tibia assessed from coronal, T2-weighted, fat-saturated images [35]. Two trained observers, who were blinded to patient characteristics as well as the sequence of images, together assessed the presence of lesions for each subject. The presence or absence of a BML was determined. The reproducibility for determination of the BML was assessed using 60 randomly selected knee MRI scans (κ value 0.88, $P < 0.001$).

Cartilage volume measurement

The volumes of the individual cartilage plates (medial and lateral tibial) were measured by two blinded assessors from the total volume by manually drawing disarticulation contours around the cartilage boundaries on each section on a workstation, as previously described [9,36], at baseline and approximately 2 years later. The CVs for the medial and lateral cartilage volume measures were 3.4% and 2.0%, respectively.

Statistical methods

Variables were assessed for normality. Age, BMI, total cholesterol, total cholesterol/HDL-C ratio and annual change in cartilage volume were all normally distributed; baseline presence and developing an incident BML compared with 'not' were binary variables. Annual change in tibial cartilage volume was calculated by subtracting the follow-up volume from the baseline volume and then dividing it by the time between MRI scans. Serum levels of triglyceride, HDL-C and LDL-C were not normally distributed and therefore the natural logarithms (ln) were used. Logistic regression was used to determine the odds of having a prevalent BML at baseline or an 'incident' BML at follow-up for each of the lipids measured. The potential confounders of age and BMI were included in the multivariate model. Linear regression was used to determine the relationship between lipids and annual change in cartilage volume. A *P* value of less than 0.05 (two-tailed) was regarded as statistically significant. All analyses were performed using the SPSS statistical package (version 15.0.0; SPSS Inc., Chicago, IL, USA).

Results

One hundred and forty-eight (84%) of the 176 eligible women completed the 2 year follow up. Apart from being younger ($P = 0.04$) there were no significant differences in BMI ($P = 0.31$), total cholesterol ($P = 0.85$), (ln)triglycerides ($P = 0.82$), (ln)LDL ($P = 0.38$), (ln)HDL ($P = 0.72$) and total cholesterol/HDL ratio ($P = 0.45$) between those who completed the follow up and those who did not. Twenty-two (15%) of the population had a BML present in their knee at baseline. One-hundred twenty-six women were BML-free at baseline. Of them, 11 (9%) developed an incident BML over the 2-year follow-up. A comparison of baseline characteristics of the 126 women (115 who did not develop a BML and 11 who did) is presented in Table 1.

Serum lipids were not found to be significantly associated with the presence of BMLs at baseline (Table 2). The relationships between serum lipids and incidence of BMLs are presented in Table 3. Incident BMLs were associated with higher total cholesterol and triglyceride concentrations but not HDL-C, LDL-C or total cholesterol/HDL-C ratio. Only one woman who developed a BML had ever smoked, so the relationship between smoking and incident BML could not be examined. The odds of developing a BML were 1.84 (95% confidence interval [CI] 1.01, 3.36) for every 1 mmol/L increase in total cholesterol after adjusting for the potential confounders of age and BMI ($P = 0.048$). The odds of an incident BML were 8.4 (95% CI 1.63, 43.43) for each unit increase in (ln)triglycerides after adjusting for confounders ($P = 0.01$). A trend between increased odds of incident BMLs and total cholesterol/HDL-C was also observed (odds ratio [OR] 1.53, 95% CI 0.98 to 2.38; $P = 0.06$) in univariate analyses, but this relationship did not reach significance after adjustment for confounders (OR 1.41, 95% CI 0.86 to 2.32; $P = 0.17$). All analyses were also performed adjusting for weight rather than BMI, but this did not alter the results (data not shown).

In addition, when all people who had a BML at either baseline or follow-up (n = 33) were grouped and compared with those who had never had a BML (n = 115), we found that the difference in BMI between the two groups approached significance with a trend of $P = 0.09$ (BML group: mean 28.8 mmol/L, SD 6.3; no BML group: mean 26.9 mmol/L, SD 5.4), but there was no difference in age or gender. Triglyceride levels were also

Table 1

Baseline characteristics of study subjects who did not develop an incident bone marrow lesion compared with those who did

	No incident BML n = 115	Incident BML n = 11	*P* value
Age, years	52.3 (6.80)[a]	54.7 (4.98)[a]	0.31[b]
Body mass index, kg/m^2	26.9 (5.42)[b]	28.9 (5.14)[a]	0.23[b]
Total cholesterol, mmol/L[c]	5.71 (3.7-8.3)[b]	6.45 (4.8-9.1)[b]	0.04[b]
Triglycerides, mmol/L[d]	1.0 (0.5-2.9)[e]	1.4 (0.8-3.8)[e]	0.01[f]
HDL, mmol/L[c]	1.5 (0.5-2.6)[e]	1.3 (0.9-2.6)[e]	0.52[f]
LDL, mmol/L[c]	3.6 (1.8-5.8)[e]	3.87 (3.2-6.3)[e]	0.19[f]
Total/HDL ratio	3.8 (2.0-7.4)[a]	5.3 (2.5-8.3)[a]	0.13[b]

[a]Mean (standard deviation or range). [b]Independent samples *t* test. [c]To convert from mmol/L to mg/dL, divide by 0.0259. [d]To convert from mmol/L to mg/dL, divide by 0.0113. [e]Median (range). [f]Mann-Whitney *U* test. BML, bone marrow lesion; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 2

Association between bone marrow lesions at baseline and lipids

	Univariate analysis, odds ratio (95% CI)	P value	Multivariate analysis, odds ratio (95% CI)[a]	P value
Total cholesterol	0.91 (0.62, 1.33)	0.63	0.9 (0.6,1.35)	0.61
Ln triglycerides	1.99 (0.77, 5.16)	0.15	1.81 (0.63, 5.20)	0.27
Ln HDL	0.45 (0.09, 2.12)	0.31	0.63 (0.11, 3.50)	0.6
Ln LDL	0.65 (0.15, 2.90)	0.58	0.58 (0.12, 2.80)	0.50
Total/HDL ratio	1.10 (0.81, 1.50)	0.54	1.03 (0.73, 1.43)	0.88

[a]Odds ratio of having a bone marrow lesion present at baseline for each unit increase in total cholesterol, natural logarithm (Ln) of triglycerides, natural logarithm of high-density lipoprotein (HDL) or low-density lipoprotein (LDL) or for the total/HDL ration after adjusting for age and body mass index. CI, confidence interval.

significantly higher in those with a BML compared with those who did not have a BML at either time point (median 1.3 mmol/L, range 0.7 to 3.8, compared with median 1.0 mmol/L, range 0.5 to 2.9; $P = 0.02$). This persisted after adjusting for age and BMI (OR 3.28, 95% CI 1.16 to 9.21; $P = 0.024$).

No association between total cholesterol, triglycerides, HDL-C, LDL-C, total cholesterol/HDL-C ratio and smoking status and annual change in total tibial cartilage volume was observed (Table 4). Similarly, when the medial and lateral compartments were analysed separately, no association was seen (data not shown).

Discussion

In this cohort study of asymptomatic middle-aged women, serum lipids were not associated with the presence of BMLs at baseline or change in knee cartilage over 2 years. However, greater levels of total cholesterol and triglycerides, even within the normal ranges, were associated with the incidence of BMLs in knees free of BMLs at baseline.

No previous study has examined the relationship between serum lipids and longitudinal change in knee structures or incident OA. There is, however, some evidence suggesting a relationship between increasing cholesterol and knee OA from cross-sectional studies [37,38]. Among patients selected based on hospitalisation for joint replacement due to advanced OA, approximately 38% had hypercholesterolemia (serum cholesterol of at least 6.2 mmol/L or on antihyperlipidemic medications) [37]. Among women from the Chingford study, moderately raised serum cholesterol levels (6.0 to 7.1 mmol/L) were associated with the presence of radiological and bilateral knee OA [38]. In our population of asymptomatic subjects with no clinically knee OA, we found no significant relationship between serum lipids and change in cartilage over 2 years.

In contrast, we found a significant relationship between serum lipids and the development of new BMLs. This finding is supported by the recent findings in a different asymptomatic population that found that dietary lipids were associated with the risk of BMLs [27]. Whilst total cholesterol and triglycerides were associated with the incidence of BMLs, no relationship was seen with the traditional vascular risk factors of total cholesterol/HDL ratio and LDL. An increased ratio indicates a higher concentration of the more atherogenic LDL compared with the HDL cholesterol and confers an increased cardiovascular risk [39]. This may be due in part to the small number of

Table 3

Association between incident bone marrow lesions and lipids

	Univariate analysis, odds ratio (95% CI)	P value	Multivariate analysis, odds ratio (95% CI)[a]	P value
Total cholesterol	1.82 (1.04, 3.2)	0.037	1.84 (1.01, 3.36)	0.048
Ln triglycerides	9.23 (2.06, 41.46)	0.004	8.4 (1.63, 43.43)	0.01
Ln HDL	0.60 (0.06, 5.99)	0.67	1.12 (0.008, 14.79)	0.93
Ln LDL	7.10 (0.56, 89.39)	0.13	5.79 (0.39,86.46)	0.20
Total/HDL ratio	1.53 (0.98, 2.38)	0.06	1.41 (0.86, 2.32)	0.17

[a]Odds ratio of having an incident bone marrow lesion for each unit increase in total cholesterol, natural logarithm (Ln) of triglycerides, natural logarithm of high-density lipoprotein (HDL) or low-density lipoprotein (LDL) or for the total/HDL ration after adjusting for age and body mass index. CI, confidence interval.

Table 4

Association between lipids and annual change in total tibial cartilage volume

	Univariate analysis, regression coefficient (95% CI)	*P* value	Multivariate analysis, regression coefficient (95% CI)[a]	*P* value
Total cholesterol	-0.24 (-12.77, 12.28)	0.97	-1.77 (-11.35, 14.88)	0.79
Ln triglycerides	0.70 (-32.48, 33.88)	0.97	12.6 (-24.12, 49.32)	0.49
Ln HDL	-4.78 (-56.43, 46.88)	0.85	-15.96 (-72.28, 40.37)	0.58
Ln LDL	3.16 (-48.06, 54.39)	0.90	13.12 (-41.2, 67.45)	0.63
Total/HDL ratio	-0.67 (-11.57, 10.24)	0.90	2.58 (-9.5, 14.68)	0.67

[a]Annual change in total tibial cartilage volume (in microlitres) for each unit increase in total cholesterol, natural logarithm (Ln) of triglycerides, natural logarithm of high-density lipoprotein (HDL) or low-density lipoprotein (LDL) or for the total/HDL ratio after adjusting for age and body mass index. CI, confidence interval.

incident BMLs reducing the power of the study to detect weaker associations, as was seen in the demonstrated relationships between incident BML and total cholesterol/HDL ratio, in which a significant univariate trend that was not persistently significant in the multivariate analyses was shown. Although we did not detect any significant associations between lipid levels and prevalent BMLs at baseline, this may be explained by the mixed nature of BMLs. To date, a number of risk factors have been identified for BMLs; one predominant risk factor is malalignment [18]. Prevalent BMLs are likely to be a diverse group and the result of a number of risk factors, including altered joint biomechanics and trauma as well as other known and unknown factors. Therefore, the ability to identify a risk factor such as serum lipids, independent of other risk factors, may be reduced among those with prevalent BMLs. In contrast, by examining those free of BMLs at baseline in this population of asymptomatic people with no clinical knee disease or symptoms, we were able to detect a relationship between serum lipids and incident BMLs. This longitudinal finding provides a much stronger level of evidence for a relationship between serum lipids and BMLs than can be obtained from a cross-sectional study [40]. Our data suggest that BMLs are not solely a consequence of biomechanical factors. It is possible that the relationship between lipids and BMLs may be a consequence of vascular pathology. Subchondral bone is highly vascularised and it has been suggested that one origin of BMLs may be ischemia and/or reperfusion injury [8]. A study of human bone marrow using gadolinium demonstrated that compared with knees free of BMLs, those with BMLs showed perfusion abnormalities, including significantly reduced venous outflow [30], and BMLs detected on MRI have been said to be similar to those seen in avascular necrosis [7].

Although some previous studies have suggested that hyperlipidemia may occur as a consequence of OA and the associated treatments, the findings of our study suggest that this is not the case but that serum cholesterol and triglycerides are positively associated with structural change in the knee in the absence of OA. Whether elevated lipid levels cause incident BMLs via a vascular mechanism is not known. Alternatively, it may be that the relationship between serum lipids and incidence of BMLs is the result of inflammatory pathways or as yet unknown mechanisms. Given that coagulation and inflammatory pathways have been shown to be intimately related [41], it is possible that the results we have observed are a combination of both effects. Histological studies in both animals and humans have demonstrated lipid, cholesterol and fibrin deposits in cancellous bone [41-43]. These lipid emboli and thrombi may result in reduced blood flow and lead to ischemia and ultimately bone necrosis. Furthermore, dogs with hip OA were shown to have increased serum lipid levels as well as evidence of hypofibrinolysis and increased platelet aggregability that could be reversed with treatment, resulting in a significant improvement in OA symptoms [41]. Thus, it may be that reducing lipids will have a beneficial effect in reducing subsequent knee OA.

Hypercholesterolemia and hypertriglyceridemia are major risk factors for cardiovascular disease in women [44]. We have found that subtle perturbations in lipid metabolism are also associated with the development of new BMLs, which are significant predictors of OA development and progression. We did not find a significant association between serum lipids and cartilage change. However, given that BMLs are associated with progression of cartilage defects [45] and loss of cartilage [19-21], it may be that our present study did not have power to show a direct relationship between serum lipids and cartilage loss. Larger studies of longer duration, especially in an asymptomatic population free of clinical OA, may be required.

This study has a number of limitations. First, the power of this study to show an effect was limited by the low number of prevalent (14%) BMLs; therefore, we were unable to examine change in BML size over 2 years. In addition, due to the modest number of subjects who developed a BML, these results will need to be confirmed in larger studies. In this study, we were not able to measure knee alignment and this may have attenuated our findings. However, recent work suggests that the relationship between BML and progression persisted after

accounting for alignment [18]. In addition, due to the low prevalence of smokers in this population, we were unable to examine how smoking may relate to loss of cartilage and incidence of BMLs. We did not obtain radiographs of the knees, so some subjects may have had asymptomatic radiographic OA. However, we used the presence of pain to exclude any potential participants who may have had clinical OA since all of the American College of Rheumatology criteria for the classification of knee OA require pain. Individuals with significant knee injury in the past, pain at baseline, knee surgery or physician diagnosis of any type of arthritis were excluded. There is debate as to how to define early knee OA since it is now clear that there is a continuum from the normal to the OA knee, with more than 10% of knee cartilage already lost by the time radiological OA is present [46]. A major strength of our findings is that serum lipids were shown to be a risk factor for incident BML among those without any BML at baseline in a population that was asymptomatic with no knee pain, no history of treatment for knee disease and no history of knee injury. This population represents an asymptomatic preclinical OA population within the spectrum of disease development and would be the population in which primary prevention measures would be used. Another potential limitation of our study is that lipids were measured 1.5 to 3.5 years prior to the MRI assessment. However, baseline measurements of lipoprotein lipids and apoproteins have been shown to be robust predictors of cardiovascular events a number of years later [47]. We also did not have information on whether women were on lipid-lowering drugs; however, the aim of this study was to examine the relationship between serum lipid levels and BMLs and cartilage. Any woman on treatment would have been classified based on her cholesterol level; therefore, those with a tendency to hypercholesterolemia but whose cholesterol was normal on assessment would have been analysed as having a normal cholesterol level. This is likely to have underestimated the effect we observed.

Conclusions

In this study of asymptomatic middle-aged women with no clinical knee OA, cholesterol and triglyceride levels were associated with the incidence of BMLs over 2 years. This provides support for the hypothesis that vascular pathology may have a role in the pathogenesis of knee OA and warrants further work to clarify this and whether treatments aimed at reducing serum lipids may have a role in reducing the burden of knee OA.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

FH, SRD, SLD and JA were involved in the design and implementation of the study, including data collection and measurement. AEW, RJB and FMC were involved in the design and implementation of the study, including data collection and measurement, and in the analysis and interpretation of the data. MD-T was involved in the analysis and interpretation of the data. All authors were involved in the manuscript preparation and read and approved the final manuscript.

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Chapter 8: General Discussion

There is no known treatment for knee OA that either prevents the development or stops the progression of the disease once it is present. Current strategies are aimed only at relieving symptoms. Ultimately costly joint replacement is the only outcome for end stage disease, identifying modifiable factors is therefore imperative.

There is evidence to suggest that the incidence and progression of knee OA may involve different mechanisms and hence preventive strategies for OA should be focused on identifying modifiable risk factors for both these processes. Therefore the aim of this thesis was to address this by examining risk factors for structural changes in the knee that are associated with either disease development or the progression of disease in both healthy/asymptomatic populations as well as in a population with knee OA. This provided the opportunity to examine these across the spectrum of disease from the normal joint through to one with OA. The findings of this thesis have the potential to further our understanding of the pathogenesis of knee OA and to identify factors that have the potential to be targets of preventative and therapeutic strategies.

8.1 Main Findings

This thesis examined the effect of biomechanical and systemic risk factors on knee cartilage and bone and their change over time in both asymptomatic/healthy subjects and in those with knee OA. Factors that affect the progression of disease, as well as those that have adverse effects on cartilage and bone in asymptomatic clinically healthy individuals were identified. Structural changes in the knee associated with the development of OA such as loss of articular cartilage, development and progression of cartilage defects, bone expansion, meniscal tears and bone marrow lesions were explored and potentially modifiable risk factors (both biomechanical and systemic) for these changes were identified. Finally, the foundations for a relationship between systemic vascular factors and knee joint structure changes have been laid, suggesting other novel preventive strategies require investigation. The results of this thesis provide a better understanding of the pathogenesis of knee OA with implications for both the prevention and early treatment of disease.

8.1.1 The Natural History of Cartilage Defects

Defects in articular cartilage are found in healthy subjects as well as in those with OA [233, 234] and have been implicated in the pathogenesis of disease [161, 163, 175, 237, 239]. While it is important to identify the factors associated with the presence of cartilage defects and their progression, there is little data on the natural history of cartilage defects in those with OA. The first paper presented within this thesis described the natural history of cartilage defects and factors associated with the progression in those with knee OA [335].

In this study, cartilage defects were found to tend to progress over 2 years in people with symptomatic knee OA. Factors associated with progression of cartilage defects were increasing age and baseline tibial bone area. Bone size has been previously identified as a risk factor for both the prevalence and severity of cartilage defects in healthy [161, 233] and OA populations [336] and more recently for the progression of cartilage defects in healthy individuals [235]. Increased bone size or expansion changes the stability of the foundation of the cartilage. The cartilage is weakened resulting in cartilage cracks and the progression of cartilage defects [233, 235, 337]. As cartilage defects are associated with cartilage loss and joint replacement [237], interventions aimed at reducing tibial bone size may have a role in reducing progression of cartilage defects and warrant further investigation.

8.1.2 The Significance of Meniscal Tears

Meniscal tears have also been identified as an important risk factor for the development and progression of knee OA [83, 338]. The second and third papers presented within this thesis described the prevalence and significance of meniscal tears with respect to knee structure, and also the relationship between gait parameters and meniscal tears in a cohort of asymptomatic women [339, 340].

Amongst pain free post-menopausal women, meniscal tears were common, especially with advancing age. Tears were also associated with greater tibial plateau bone area, and trends between prevalence of meniscal tear at baseline and decreased lateral cartilage volume as well as with the progression of the

tibiofemoral cartilage defects over 2 years were also found. This study thus provides support to the hypothesis that tibial plateau bone changes occur before significant pathological changes in cartilage. The question that remains is whether increased tibial plateau bone area predisposes to an increased risk of degenerative meniscal tears or whether it is a consequence of altered biomechanical forces in relation to meniscal tear.

The second paper in this section, demonstrated that gait parameters that indicate medial tibiofemoral joint loads are associated with medial meniscal pathology. The presence and severity of medial meniscal tears was positively associated with the peak external knee adduction moment during early stance, and tended toward a similar association during late stance. In addition, the presence of medial meniscal lesions was also positively associated with the degree of internal foot rotation when the external knee adduction moment peaked during late stance, independent of the magnitude of the adductor moment. This was the first study to describe an association between gait parameters and meniscal tears. The possibilities are that either meniscal lesions predict aberrations in gait or, alternatively, that the gait parameters contributed to the development of these lesions. Since meniscal tears are associated with the structural changes of OA, including cartilage defect scores, reduced tibial cartilage volume and increased tibial bone area [83, 128, 179, 182, 184, 185, 330], it is possible that modifying the gait parameters examined in this study (e.g. via gait retraining or orthoses) may also help to reduce the incidence and burden of knee OA.

8.1.3 Local Biomechanical Factors May Explain Compartmental Effects

There is a general consensus that one of the leading risk factors for OA is joint biomechanics [76]. Although the disease can occur at any site within the knee joint, the medial compartment is the most common site of tibiofemoral involvement while the lateral patella femoral joint is most commonly affect in patella-femoral disease [34-38]. Is has been suggested that local biomechanical factors leading to differing compartmental loading may explain these differing findings. Joint mechanics however are not fixed and it is unclear whether changes in knee alignment are associated with change in knee cartilage volume within the context of disease. Correcting or attenuating malalignment may potentially be a target for influencing disease

progression yet very little is known. The fourth and fifth papers presented within this thesis explore local biomechanical factors affecting the tibiofemoral and patella femoral compartment.

In people with knee OA, a change in knee alignment from genu varum toward genu valgum over 2 years was associated with a reduction in the annual rate of medial tibial cartilage volume loss among people with knee OA in the subsequent 2.5 years. Change in alignment did not affect the rate of change in lateral tibial cartilage volume. These findings suggest that methods to reduce varus alignment may delay the progression of medial tibiofemoral OA. The current study adds to previous knowledge by providing the first evidence that *change* in alignment away from genu varum toward genu valgum is associated with a reduction in the subsequent rate of medial tibial cartilage loss, without increasing the rate of lateral tibial cartilage volume loss in people with knee OA. This infers that minor changes (e.g., 1°) away from genu varum, which ultimately reduces the static load exerted to the medial tibiofemoral compartment, can reduce the rate of cartilage loss in that compartment.

Within the patella-femoral compartment, it was demonstrated that among people with knee OA, a more shallow femoral sulcus angle was associated with increased medial patella cartilage volume compared to a deeper femoral sulcus angle. For every 1° increase in the femoral sulcus angle (i.e., as the sulcus became more shallow), there was an associated 9.1 mm^3 increase in medial patella cartilage volume among people with knee OA. Despite the cross-sectional association, the femoral sulcus angle at baseline was not associated with longitudinal change in patella cartilage volume over 2 years. No previous study has examined the relationship between the femoral sulcus angle and patella cartilage volume. These data suggest that a shallower sulcus in the context of established OA may be an advantageous anatomical variant. Further longitudinal studies are required to elucidate the role the femoral sulcus angle plays in OA.

8.1.4 The Natural History and Significance of Bone Marrow Lesions

There is increasing interest on the role of BMLs, detected by MRI, in the pathogenesis of knee OA [41, 78]. While BMLs have been shown to be present in both symptomatic [41, 172, 174-177, 239, 270, 278] and asymptomatic populations [171,

173], most previous studies have focussed on symptomatic populations with established knee OA [41, 172, 174-177, 239, 270, 278]. There is very little information however, about the role of BMLs in healthy asymptomatic populations. The sixth and seventh papers presented within this thesis described the natural history and significance of bone marrow lesions in those with no clinical knee OA.

In a population of healthy participants without clinical knee OA and free of BMLs at baseline, BMLs developed in 12% of people over 2 years. Increased weight and BMI were risk factors for incident BMLs. Incident BMLs were also associated with the development of knee pain in a population where all participants were free of pain at the beginning of the study. Approximately half of the BMLs present at baseline resolved over the 2 year study period. This is the first study to report the natural history of BMLs and risk factors associated with the incidence of BMLs in a healthy, pain free population. The findings of this study suggest that BMLs occur in healthy populations but are less common and are more likely to revolve compared to OA/symptomatic populations [239, 270, 271, 278]. In this current study, the incidence of BMLs was also associated with developing new knee pain, mirroring previous observations in OA/symptomatic populations. This suggests that BML play a role in knee pain in OA possibly as a continuum from a normal to a clinically diseased joint. These data suggest that in middle aged people with no clinical knee OA, BML are reversible and may provide a target for interventions aimed at symptoms and prevention of knee OA.

In addition within this asymptomatic population, the development of new BMLs was associated with adverse effects on knee cartilage, while resolution of BMLs was associated with less deterioration of cartilage. It may be that in the observed relationship between BMLs and cartilage, factors contributing to the development of BMLs also impair the supply of nutrients and oxygen to the overlying cartilage plate as well as reducing the strength of the bony foundation of articular cartilage [274-276] Our data also suggest that the effect of BMLs may be reversible since their resolution was associated with less cartilage loss and likelihood of defect progression. Thus identifying factors that reduce the incidence of BMLs and increase their resolution may offer therapeutic targets in the prevention of knee OA.

8.1.5 Systemic Risk Factors for Bone Marrow Lesions

Given the significance of BMLs and their role in knee OA, understanding modifiable factors which may influence their development is important. Histological examination of BMLs in knees has shown that they may represent areas of osteonecrosis, oedema, trabecular abnormalities and bony remodeling [170]. While originally thought to predominantly be a result of joint mal-alignment [78] there is increasing evidence to suggest that they may also be associated with systemic or vascular factors [279, 289, 291]. The final 3 papers within this thesis examined the relationships between cigarette smoking, dietary fatty acids and serum lipids and bone marrow lesions in an asymptomatic clinically healthy population.

In a cohort of asymptomatic, community based adults, a history of smoking (current and past) was associated with increased medial tibial, but not lateral tibial or patella cartilage loss over two years. In addition there was a dose-response relationship between 'pack-years' smoked and increased medial tibial cartilage loss. For individuals who had a BML at baseline, smoking was also associated with the persistence of the BML over two years. The persistence of the BML was found to partially mediate the relationship between smoking and cartilage loss. The results from this study provide a possible mechanism for the previously observed negative association of smoking with structural knee changes associated with development of knee OA and suggest that the association smoking shares with increased cartilage loss may be mediated via smoking impairing the ability for BMLs to resolve.

In the same population a higher intake of saturated fatty acids, was found to be associated with an increased likelihood of developing BMLs over 2 years. This was the first longitudinal study presenting a relationship between dietary fatty acid intake and the incidence of BMLs. It has been suggested that subchondral ischaemia may be a mechanism by which vascular pathology plays a role in the initiation and/or progression of OA [274]. As subchondral bone is important in maintaining joint integrity and the development of OA, this study suggests that dietary modification of fatty acid intake may be one strategy in the prevention of knee OA that warrants further investigation.

In asymptomatic middle-aged women with no clinical knee OA, cholesterol and triglyceride levels were associated with the incidence of BMLs over 2 years. No previous study has examined the relationship between serum lipids and longitudinal change in knee structures. This data further suggests that BMLs are not solely a consequence of biomechanical factors. The results of this study provide support for the hypothesis that vascular pathology may have a role in the pathogenesis of knee OA and warrants further work to clarify this and whether treatments aimed at reducing serum lipids may have a role reducing the burden of knee OA.

8.2 Potential Limitations of this work

8.2.1 Populations Examined

The results of this thesis are based on four different populations. The first population is comprised of symptomatic men and women with knee OA and therefore the results of these studies cannot be generalised to healthy subjects. To extend the findings and explore OA pathogenesis in the pre-OA stages, 3 asymptomatic populations were examined. One was comprised of middle-aged women who were free of radiographic knee OA, the second an asymptomatic clinically healthy community based adults selected from a cohort study and the third a second cohort of middle-aged women who were asymptomatic and clinically free of knee OA taking part in a different cohort study. Potential limitations of these populations are that they consist of people willing to take part in studies so may represent a more health conscious group. However it is unlikely that such bias will significantly affect the association between risk factors and knee structure.

Gender limitation applied to two of the cohorts examined in this thesis, both of these studies were restricted to women only. Gender is a significant confounder and by restricting the results to women only we have been able to reduce the effect. The results from these cohorts however cannot be generalised to men.

For all populations examined participants were aged over 40. As a result all populations examined were predominantly middle-aged. The ages of participants in

the population of symptomatic knee OA were older again. While this age restriction limits generalisations to these age profiles, it is also a strength as it reduced any effects of this significant confounder. However further work will need to be done across the spectrum of aged to determine whether similar relationships exist and whether some relationships are age dependent.

8.2.2 Study Design

8.2.2.1 Cross-sectional Studies

Some of the results presented in this thesis were based on cross-sectional studies. The results from those studies were unable to determine a temporal relationship between risk factor and outcome. Longitudinal studies are required to confirm those results.

8.2.2.2 Longitudinal Studies

Loss to follow up may have introduced selection bias to the longitudinal studies presented within this thesis however for the four populations examined the follow up rate was good (>80%). In addition a comparison of baseline characteristics of those who did and those who did not complete the study was done for each of the populations and the characteristics of those who completed the study tended to be similar.

Secondly, the two year follow up employed in 3 of the 4 populations examined in this thesis may not have allowed sufficient time to determine the relationships between the specific risk factors and changes in knee structure. Follow-ups of longer duration may be required.

8.2.3 Measurement of Risk Factors

8.2.3.1 Measurement of Smoking Status

The measurement of smoking status and history, whilst determined in a valid fashion was based on the participants self-report and is therefore associated with recall bias.

Furthermore, our classification of 'ever smoked' may have introduced some misclassification bias, which may have underestimated the associations between smoking and cartilage and BMLs rather than overestimated them.

8.2.3.2 Measurement of Dietary Intake

Whilst the dietary intake of fatty acids was measured in a valid fashion [341], this was based on a single measure of nutrient intakes 10 years earlier. Although significant underreporting of fat intake is likely [342], absolute intake of dietary fat appears to have remained stable [343, 344]. While nutritional data collected 10 years earlier may have resulted in some misclassification of exposure, such misclassification is likely to have been non-differential in relation to knee structure, since only subjects with no history of knee symptoms or injury were included thereby tending to underestimate the strength of any observed associations.

8.2.3.3 Measurement of Blood Lipids

Lipids were measured 1.5-3.5 years prior to the MRI assessment. However baseline measurements of lipoprotein lipids and apoproteins have been shown to be robust predictors of cardiovascular events a number of years later [345]. We also did not have information on whether women were on lipid lowering drugs. Any woman on treatment will have been classified based on her cholesterol level, therefore those with a tendency to hypercholesterolemia, but whose cholesterol was normal on assessment, will have been analysed as having a normal cholesterol level. This is likely to have underestimated the effect we observed.

8.2.4 Measurement of Knee Structure

8.2.4.1 Radiographs

For two of the populations examined we did not obtain radiographs of the knees. It is possible that some subjects may have had asymptomatic radiographic OA. We used the American College of Rheumatology clinical criteria of OA [1] to determine the status of knees. Individuals with significant knee injury in the past, pain at baseline,

knee surgery or physician diagnosis of any type of arthritis were excluded. There is debate as to how to define early knee OA since it is now clear that there is a continuum from the normal to the OA knee with more than 10% of knee cartilage already lost by the time radiological OA is present [130]. It is likely that these populations represent an asymptomatic, pre clinical OA population, within the spectrum of disease development and would be the population in which primary prevention measures would be used.

8.2.4.2 Magnetic Resonance Imaging

The methods used to measure cartilage volume, cartilage defects, meniscal tears, subchondral bone and bone marrow lesions from MRI have been shown to be valid and are associated with excellent reproducibility with; coefficients of variation of 2-3% for cartilage volume and bone area [142, 169]; intraclass coefficients of 0.89-0.94 and 0.86-0.96 for cartilage defects and meniscal tears respectively [128, 217]: and a kappa value of 0.88 for bone marrow lesions [171, 173]. In addition, extra care was taken in training individual observers and all measures were performed over a short period time by one main observer with a second checking 1 in 5, and all results sent to a third independent person. Initially, the main observer was trained by an experienced person and actual measurements did not start until measures were within 10% of the trainer and also when repeated against the trained observer himself. All measures were then sent blindly to a third person who checked them and advised whether the measures should continue. Regular calibration between the trainer and the observer also took place weekly to ensure reproducibility and reliability.

For longitudinal studies, all baseline and follow-up scans were re-measured at the same time to avoid systemic bias.

In order to minimise information bias; outcome measures were entered in the database using ID numbers only and all forms of measurement were performed without the knowledge of personal details of the individual.

All MRI's were read blindly to reduce the likelihood of differential misclassification.

8.2.5 Data Analysis and Interpretation

8.2.5.1 Sample sizes

The study examining meniscal tears and gait was based on a small cohort of 20 middle-aged women. While we did have sufficient power to detect a relationship between biomechanical parameters and the presence and severity of meniscal tears, we may have been unable to detect weaker relationships.

The studies examining BMLs were limited by the low prevalence and/or incidence of BMLs in the respective populations and therefore we may not have been sufficiently powered to detect weak associations between risk factors and BMLs, nor did we have the power to examine changes in BML volume. Nevertheless we were able to demonstrate significant relationships between BML incidence and persistence or resolution, risk factors and pain symptoms.

8.2.5.2 Determining causation

Further studies are required to determine the relationships between the risk factors and outcomes examined. In this study cartilage, bone and the menisci were examined. It is possible that other structural elements within the knee that can be visualised using MRI including synovial and ligament changes may influence the development and/or progression of OA. They however were not examined within this thesis. Further work is required to assess their role. In addition the results presented within this thesis are from observational studies, ideally, where possible, large randomised controlled trials are required to confirm the results.

8.3 Future Directions

OA is a significant public health problem. It is the major cause of disability and imposes a huge economic burden to the community. The prevalence of OA is expected to increase given the current obesity epidemic and ageing of the population.

Identifying modifiable factors that influence either the progression and/or development of disease is of particular importance.

The use of MRI in knee OA has allowed a new approach to understanding the pathogenesis of the disease and the identification of risk factors. Not only can articular cartilage be assessed, but also other structural components of the knee including subchondral bone, meniscal pathology, ligaments and synovial changes. For the first time potential structural biomarkers of disease development and/or progression can be identified. It is also possible to assess the joint as a whole and examine the inter-relationship between the different structural components. The joint can be explored as a continuum from a healthy, asymptomatic joint, through to one with early changes of knee OA and then subsequently to the end stage joint. How a certain risk factor or structural element behaves in osteoarthritic populations may be different to how it does in healthy populations since the onset of early disease and symptoms may modify the effect of a given risk factor. Given that there is no cure for OA or treatment that slows progression future work is required to increase the understanding of these interactions in different population types. This will allow a better understanding of the pathogenesis of knee OA and provide novel approaches to the prevention and treatment of knee OA.

Cartilage defects are commonly found in healthy subjects and those with knee pain or OA. They are associated with pain, radiographic features and severity of OA. In people with knee OA they tend to get worse. Given that they predict both cartilage loss and joint replacement, understanding factors associated with their progression may allow the development of strategies to slow the progression of disease. Further work is required to identify these modifiable factors. In addition as we found that subchondral bone size predicted cartilage defect progression, interventions aimed at reducing tibial bone size may have a role in reducing progression of cartilage defects and warrants further investigation.

Meniscal tears have also been associated with the pathogenesis of knee OA. While originally described in relation to trauma, tears are also a product of degenerative processes. Work examining meniscal tears has predominantly been performed in OA populations and there is very limited research in pre-OA, despite them being shown to be common in these populations. Further work is required examining risk factors for degenerative tears in asymptomatic populations, as is the relationships between meniscal tears and structural changes prior to disease development. The results presented within this thesis suggest that biomechanical factors are associated with both the presence and severity of tears, the findings are however based on cross-sectional data and longitudinal studies are required to further describe the direction of the relationship.

To date most research has examined the tibiofemoral compartment and yet the symptoms of knee OA have been shown to predominantly emanate from the patellofemoral compartment. One of the leading risk factors for OA is the altered joint biomechanics which may influence joint geometry and load distribution with in the joint. The effect of biomechanical factors on the knee joint may have very different effects on each compartment and depending on whether disease is present in neither, either or both compartments. Further work is needed to examine these possible relationships. As demonstrated within this thesis joint biomechanics are not static and attenuating or trying to correct joint biomechanics may be yet another approach to preventing the development and/progression of knee OA.

It is widely accepted that bone is integral to the pathogenesis of OA. Changes in subchondral bone have been well described in established OA. These changes affect the mechanical properties of the subchondral bone and have been proposed to play a role in the initiation and progression of degeneration of the overlying articular cartilage. While it remains unclear and controversial whether cartilage or bone acts as the instigating lesion of OA, there is growing evidence for the significant role of BMLs. We showed that in asymptomatic individuals they are reversible. Given their role in the pathogenesis of OA, further work identifying factors associated with their development, progression and improvement is needed.

The emerging evidence supports the notion that OA has multiple aetiologies, which converge to produce the recognized manifestations of joint pain and stiffness and degeneration of articular cartilage and the end stage joint. However, a lack of understanding of the underlying cause(s) for OA has meant that treatments remain largely palliative, with joint replacement the only option in end-stage disease. Exploring the possible associations' vascular pathology has with structural changes in the knee associated with disease development and progression has the potential to provide an alternative approach to understanding the pathogenesis of knee OA which may lead to novel preventive strategies. Given the highly vascularised nature of bone further work exploring the effects of systemic factors on subchondral bone, in particular bone marrow lesions is desirable and has implications for the prevention and early treatment of disease.

This thesis has contributed to the identification of knee structural changes in both the pre-diseased and diseased state as well as risk factors for these changes. Further work will be required to better understand the role of these different structural changes in the early disease and their associated risk factors in order to more effectively prevent and treat knee OA.

Chapter 9: Conclusions

This thesis examined the relationship between risk factors for knee OA and knee structure. It examined:

1) Effect of biomechanical and systemic risk factors on knee cartilage, meniscal tears and bone and the significance of their change over time in both symptomatic/healthy subjects and in those with knee OA. It identified a number of modifiable factors that influence changes indicative of disease development as well as disease progression.

2) In subjects with knee OA understanding factors that influence the progression of cartilage defects as well as correcting joint biomechanical features may be a possible target for slowing the progression of disease.

3) In pre-disease/asymptomatic populations, the significance of meniscal tears was explored and the role of gait parameters was examined. The results suggested that meniscal tears may increase a person's risk of developing knee OA via tibial plateau expansion, and that modification of gait parameters may be a potential target in the prevention of knee OA.

4) The natural history of BMLs in an asymptomatic community based cohort was examined and risk factors associated with the development, persistence and resolution of BMLs was explored.

5) Finally, this thesis has laid the foundations for a relationship between systemic vascular factors and knee joint structure changes, suggesting other novel preventive strategies.

The results of this thesis provide a better understanding of the pathogenesis of knee OA by examining different cohorts with implications for both the prevention and early treatment of disease.

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Appendices

Manuscripts not directly related to thesis

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Bone marrow lesions predict progression of cartilage defects and loss of cartilage volume in healthy middle-aged adults without knee pain over 2 yrs

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Objective. In knee OA, the presence of bone marrow lesions (BMLs) predicts pain and progression of disease. Their occurrence has been described in healthy, pain-free subjects, but whether their presence affects change in cartilage is unknown.

Methods. Two hundred and seventy-one healthy community-dwelling adults with no history of knee injury, knee pain or clinical knee OA had an MRI performed on their dominant knee at baseline and 2 yrs later to assess the relationship between the presence of BMLs at baseline and change in tibiofemoral cartilage defects and tibial cartilage volume over 2 yrs.

Results. BMLs were present in 37 (14%) subjects. Cartilage defects were more likely to progress rather than remain stable or regress in subjects with BMLs compared with those without BMLs ($P = 0.04$). The odds of cartilage defects progressing in the tibiofemoral compartment of the knee where BMLs were present compared with where BMLs were absent was 2.6 (95% CI 1.2, 5.3; $P = 0.01$). Where 'very large' BMLs were present, there was a trend for increased annual tibial cartilage volume loss (46.4 mm^3/yr; $P = 0.07$).

Conclusions. These data suggest that BMLs are associated with change in knee cartilage over 2 yrs in asymptomatic subjects. Increased progression of cartilage defects is seen with increasing size of BMLs. It will be important to determine in future studies whether BMLs directly cause change in cartilage over 2 yrs, or act as a marker of another factor that facilitates these changes.

KEY WORDS: Osteoarthritis, Cartilage, Bone marrow lesions, Cartilage defects, Cartilage volume.

Introduction

There is an increasing interest in the role of bone marrow lesions (BMLs) in the pathogenesis of knee OA [1, 2]. In knee OA, the presence of BMLs has been related to increased cartilage loss, measured by biomarkers and using MRI [3, 4]. Recently, the presence of BMLs in healthy populations without knee pain or a history of significant knee trauma has been described [5, 6]. Whether the presence of BMLs in healthy, pain-free subjects is associated with effects on cartilage is unknown. Previous studies have been performed to examine this relationship only in subjects with OA or at high risk for OA [1, 2].

In healthy adults without symptomatic or established radiographic knee OA, the early structural changes of knee OA may be present [7–9]. In middle-aged adults without knee pain, there is a tendency for cartilage defects to develop and progress, as well as cartilage volume to be lost [10, 11]. It is likely that as these changes progress, the risk of knee OA increases. In established knee OA, these changes predict pain and joint replacement [12–14]. Identifying factors that relate to change in cartilage may be important in preventing knee OA.

We performed a longitudinal cohort study to assess the relationship between the presence of a BML at baseline and change in cartilage defects and cartilage volume in healthy adults, without knee pain or a history of significant knee trauma.

Subjects and methods

Subjects

The study was conducted within the Melbourne Collaborative Cohort Study (MCCS), a prospective cohort study of 41 528 people, assembled to examine the role of lifestyle and genetic factors in the risk of cancer and chronic diseases in Melbourne, Australia [15]. Participants for the current study were recruited from this cohort in 2003–04, as described [16]. Briefly, participants were eligible if they were aged between 50 and 79 yrs without any of the following exclusion criteria: a clinical diagnosis of knee OA as defined by American College of Rheumatology criteria, which require the presence of pain [17]; knee pain lasting for >24 h in the last 5 yrs; a previous knee injury requiring non-weight-bearing treatment for >24 h or surgery (including arthroscopy); a history of any form of arthritis diagnosed by a medical practitioner or a contraindication to MRI [16]. Radiographs were not obtained, hence it is unknown whether participants had radiographic OA. The study was approved by The Cancer Council Victoria's Human Research Ethics Committee and the Standing Committee on Ethics in Research Involving Humans of Monash University. All participants gave written informed consent.

Anthropometric data

Height (in centimetres) was measured using a stadiometer with shoes removed at MCCS baseline (1990–94). Weight (in kilograms) was measured with bulky clothing removed at the time of baseline MRI. BMI was calculated from these data [weight (kg)/height^2 (m^2)].

MRI and the measurement of cartilage volume, defects and BML

MRI. An MRI of the dominant knee of each participant was performed between October 2003 and December 2004 and ~2 yrs later, as described on a 1.5-T whole body MRI unit (Phillips, Eindhoven, Holland) [18]. The following sequence and parameters were used on both occasions: a T_1-weighted fat-suppressed 3D gradient recall acquisition in the steady state; flip angle 55°; repetition time 58 ms; echo time 12 ms; field of view 16 cm; 60 partitions; 512 × 512 matrix; and one acquisition time of 11 min 56 s. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31 × 0.31 mm^2 (512 × 512 pixels). In addition, a coronal T_2-weighted fat-saturated acquisition, repetition time 2500–3000 ms, echo time 40 ms, with a slice thickness of 3.0 mm, a 0.3 mm inter-slice gap, 1 excitation, a field of

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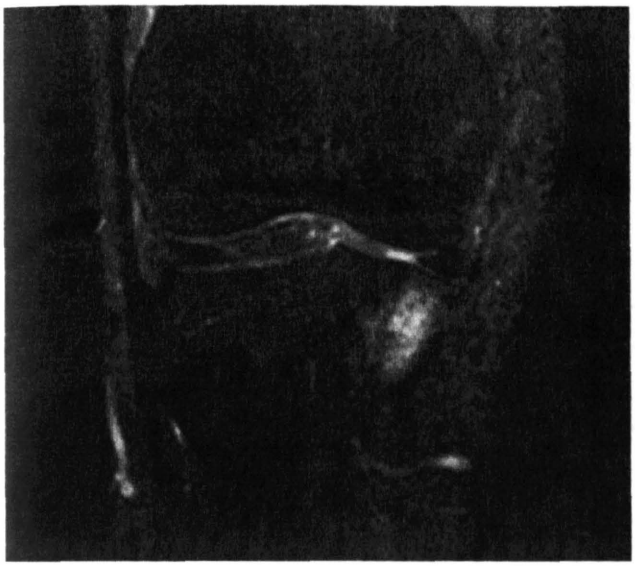

FIG. 1. A T_2-weighted image of a very large medial tibial BML.

view of 11–12 cm and a matrix of 512 × 512 pixels was also obtained [18].

Assessment of BMLs. BMLs were defined as areas of increased signal intensity immediately underlying subcortical bone in either the medial or lateral distal femur or proximal tibia on T_2-weighted coronal images [19]. Two trained observers, who were blinded to patient characteristics, as well as image sequence, together assessed the presence of BMLs for each subject [1]. The presence or absence of BMLs was determined. A BML was defined as 'large' if it appeared on two or more adjacent slices and encompassed at least one-quarter of the quadrant of the tibial or femoral cartilage being examined from coronal images [1, 20]. This is comparable with the previously described 'Grade 2' BML by Felson [1, 20]. A BML was further classified as 'very large' if it appeared on three or more adjacent slices (Fig. 1) [1]. This is comparable with the previously described 'Grade 3' BML by Felson [1, 20]. The reproducibility for determination of BMLs was assessed using 60 randomly selected knee MRIs (κ-value 0.88; $P < 0.001$).

Assessment of cartilage defects. Cartilage defects were graded on the T_1-weighted sagittal MR images with a classification system that has been previously described [9, 14, 21], in the medial and lateral tibial and femoral cartilages (Fig. 2). Intra-observer reliability (expressed as intra-class correlation coefficient, ICC) was 0.90 for the medial tibiofemoral compartment and 0.89 for the lateral tibiofemoral compartment [22]. Change in cartilage defects in a compartment was classified as to whether or not they progressed (i.e. an increase in cartilage defect score), regressed (i.e. a reduction in cartilage defect score) or remained stable (i.e. no change in cartilage defect score).

Cartilage volume measurement. The volumes of the individual cartilage plates (medial and lateral tibial) were measured from the total volume by manually drawing disarticulation contours around the cartilage boundaries on each section on a workstation as described on the T_1-weighted sagittal images [16]. The coefficients of variation (CVs) for the medial and lateral cartilage volume measures were 3.4 and 2.0%, respectively [8, 23]. Annual change in cartilage volume was calculated as: (follow-up cartilage volume subtracted from initial cartilage volume) divided by the period of time between MRI scans, as described [23].

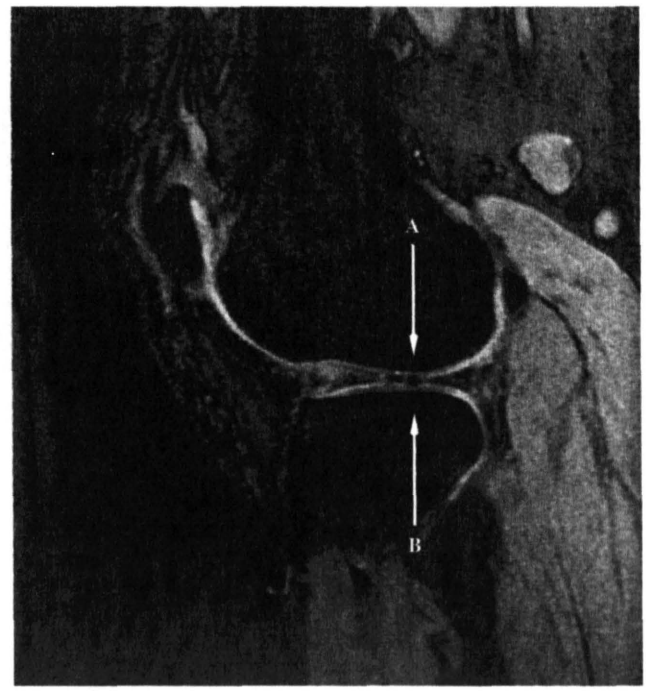

FIG. 2. T_1-weighted MRI of Grade 3 femoral cartilage defect (A) and Grade 2 tibial cartilage defect (B).

Statistical methods

Baseline characteristics were compared between subjects in whom BMLs were present and absent, using unpaired *t*-test for continuous variables, chi-square for dichotomous variables and eta test for ordinal variables. Ordinal regression was used to examine the likelihood of defect progression, remaining stable or regressing according to whether a BML was present. Change in cartilage defects was described as progression or not (including remaining stable or regressing). Logistic regression was used to determine the odds of cartilage defect progression *vs* regression/stability depending on the presence of a BML, and to adjust for potential confounding. After the distribution of annual change in cartilage volume was examined for normality, linear regression techniques were used to examine the relationship between BML and change in cartilage volume adjusting for potential confounding. Analysis of residuals was performed to exclude non-linearity. A *P*-value <0.05 (two-tailed) was regarded as statistically significant. All analyses were performed using the SPSS statistical package (version 15.0.0, SPSS, Cary, NC, USA).

Results

Two hundred and ninety-seven subjects entered the study, 271 (91%) of whom completed the follow-up MRI. The baseline characteristics of the 271 subjects with and without BMLs at baseline are compared in Table 1. There were no significant differences between these groups. The reasons for loss to follow-up for 26 subjects were death ($n = 3$), poor health ($n = 4$), withdrawal of consent ($n = 10$), ineligible for follow-up ($n = 4$) and being unable to be contacted ($n = 5$). The characteristics of subjects who were lost to follow-up tended to be similar to those who completed the study apart from their tendency to overweight [mean BMI 27.9 kg/m^2 (S.D. 5.4)], compared with those who completed the study [mean BMI 25.7 kg/m^2 (S.D. 4.1); $P = 0.01$ for difference], and to have very large BMLs (4 'very large' BMLs; $P = 0.01$).

BMLs were present in 37 subjects (13.7%), comprising of 26 large BMLs and 11 'very large' BMLs. There were 25 large BMLs in the medial compartment, of which 6 were 'very large'. In the

TABLE 1. Characteristics of participants with and without BMLs at baseline

	BMLs at baseline ($n=37$)	No BML at baseline ($n=234$)	*P*-value[a]
Women (%)	24 (64.9)	145 (62.0)	0.74[b]
Age (yrs)	58.2 (5.9)	57.8 (5.2)	0.60
Weight (kg)	73.5 (12.1)	72.8 (14.1)	0.28
Height (cm)	169.4 (7.5)	167.9 (9.2)	0.77
BMI (kg/m^2)	25.7 (4.3)	25.8 (4.1)	0.93
Total tibial cartilage volume (ml)	3.84 (1.00)	3.74 (1.11)	0.61
Total tibiofemoral cartilage defect score[c]	3 (3, 10)	3 (2, 11)	0.10[c]

Values expressed as mean (S.D.), except where indicated. [a]Unpaired *t*-test for difference, except where indicated. [b]Chi-squared test. [c]Expressed as median (range), difference examined using eta test.

TABLE 2. The outcome of total tibiofemoral cartilage defects, according to baseline cartilage defect score and presence or not of a large BML at baseline

Baseline defect score	Large BML present			No large BML present		
	Progress	Stable	Regress	Progress	Stable	Regress
2	0	0	0	12	5	0
3	4	1	0	21	13	1
4	5	4	0	17	37	3
5	5	2	0	19	28	9
6	1	3	1	7	17	2
7	2	1	1	7	10	7
8	2	2	1	3	6	2
9	1	0	0	2	1	1
10	1	0	0	0	3	0
11	0	0	0	1	0	0

TABLE 3. The outcome of total tibiofemoral cartilage defects, according to baseline cartilage defect score and presence or not of a very large BML at baseline

Baseline defect score	Very large BML present			No very large BML present		
	Progress	Stable	Regress	Progress	Stable	Regress
2	0	0	0	12	5	0
3	1	0	0	24	14	1
4	2	1	0	20	40	3
5	3	0	0	21	30	9
6	1	1	0	7	19	3
7	0	0	0	9	11	8
8	1	1	0	4	7	3
9	0	0	0	3	1	1
10	0	0	0	1	3	0
11	0	0	0	1	0	0

TABLE 4. Proportion of cartilage defects progressing (deteriorating), remaining stable or improving (regressing) depending on whether a large or very large BML was present at baseline[a]

	Large BML at baseline			Very large BML at baseline		
	BML ($n=37$)	No BML ($n=234$)	*P*-value[a]	BML ($n=11$)	No BML ($n=260$)	*P*-value[a]
Change in cartilage defects						
Progress/deteriorate	21	89		8	102	
Stable/no change	13	120		3	130	
Regress/improve	3	25	0.04	0	28	0.05

[a]Ordinal regression.

lateral compartment, there were 14 large BMLs, of which 5 were 'very large.' Two subjects had a BML in both the medial and lateral compartments.

Whether in a knee total baseline cartilage defect progressed, remained stable or regressed according to whether or not a large or very large BML was present, and according to the initial severity of cartilage defects, is presented in Tables 2 and 3, respectively. We examined the relationship between change in cartilage defects (comparing those which progressed/deteriorated with those that remained stable and those that showed improvement/regressed) and the presence of a BML at baseline using ordinal regression (Table 4). There was a tendency for those with a BML to show progression/deterioration of cartilage defects rather than for them to remain stable or to show improvement/regress compared with subjects where no BML was present initially ($P=0.04$ where a large BML was present and $P=0.05$ where a very large BML was present).

The odds of cartilage defect progressing were examined according to whether or not a BML was present at baseline (Table 5). Where a 'large' or 'very large' BML was present in the knee, cartilage defects were more likely to progress in both univariate and multivariate analyses, compared with where no BML was present, adjusting for the potential confounders of age, gender, BMI and initial cartilage defect score ($P=0.01$–0.04, Table 5). When these relationships were examined in the medial and lateral compartments individually in multivariate analysis, whilst the direction of effect was the same throughout, the results only reached statistical significance in the lateral compartment (large BML $P=0.003$, very large BML $P=0.03$).

The relationship between the presence of a BML and annual change in total tibial cartilage volume was examined. In univariate analysis, the presence of a 'very large' BML trended towards predicting increased annual tibial cartilage loss (49.5 mm^3/yr; $P=0.07$). After adjusting for the potential confounders of age, gender, BMI and initial cartilage volume, although the direction of

TABLE 5. Odds ratios of progression of cartilage defect score depending on whether any BML or a large BML is present

	Univariate odds ratios	*P*-value	Multivariate odds ratios[a]	95% CI	*P*-value
Odds of progression of total tibiofemoral cartilage defect score					
Any BML	2.14	0.03	2.56	1.23, 5.31	0.01
Very Large BML	4.13	0.04	4.88	1.24, 19.11	0.02

[a]Adjusted for age, gender, BMI and initial cartilage defect score.

effect remained, the significance of this result diminished (regression coefficient 39.4 mm^3/yr; 95% CI −13.0, 91.7; $P=0.14$). When these relationships were examined in the medial and lateral compartments individually, whilst the direction of effect was similar in both the compartments it only reached statistical significance in the lateral compartment (data not shown).

Discussion

These data suggest that the presence of a BML in knees of adults without knee pain or history of significant trauma is associated with cartilage changes over 2 yrs. In tibiofemoral compartments in which a 'large' or a 'very large' BML was present, cartilage defects were more likely to progress rather than remain stable or regress, and the odds of cartilage defect progression were increased. The odds of progression increased with increasing BML size. Where a 'very large' BML was present, there was a trend towards increased annual tibial cartilage volume loss compared with where no BMLs were present.

No previous studies have examined how the presence of a BML relates to change in cartilage in subjects without painful knee OA. In healthy subjects, where a BML was present, cartilage defects were also more likely to be present [6]. Previous longitudinal studies have examined this relationship in subjects with knee

OA [3, 4, 20]. In established OA, where a BML was present there was increased cartilage loss, as measured by MRI and also by using biomarkers [3, 4]. With increased size of BMLs, increased cartilage loss was seen in established OA [3]. The current study suggests that where a BML is present in a person without knee pain or significant trauma, knee cartilage is also more likely to be lost and that with increasing size of a BML, the risk of cartilage defect progression is increased. These data suggest that BMLs lie on one of the pathways of progression of the structural changes in knee OA. However, we cannot determine whether BMLs directly cause the cartilage changes or this is an indirect effect, and their presence is merely a surrogate marker for another causative factor.

In either case, it is possible that targeting factors that affect the presence of BMLs may also affect change in cartilage and the possibility of progression to knee OA. Bone may be a more responsive target in prevention of OA than cartilage, since known risk factors for knee OA such as knee adduction moment have been shown to affect bone before cartilage changes are present in healthy people [24]. In contrast, previous interventions aimed to affect change in cartilage directly have been largely unsuccessful [25, 26]. This approach appears promising, since use of osteoprotective therapy has been associated with reduced prevalence of BMLs [27]. In OA, use of bisphosphonates has been shown to reduce cartilage metabolism as measured by biomarkers, as well as to diminish the trabecular changes of OA in subchondral bone whilst reducing progression of cartilage loss over 2 yrs [28, 29]. Thus, it may be important to further characterize whether the relationship between BMLs and cartilage change is direct or indirect, with a view to disease prevention.

This study has a number of limitations. There were few BMLs at baseline in this healthy population. Nevertheless, we have demonstrated that even in this population the relationship between the presence of a BML and change in cartilage exists. Although the relationship between the presence of a BML and change in cartilage volume did not attain statistical significance, the direction was consistent with change in defects. This relationship may require a larger sample size or a longer duration of follow-up as we may not have had enough power to show this relationship. Since the number of participants with BMLs was low, our ability to demonstrate compartmental changes was also limited. However, the direction of effect seen in the total tibiofemoral compartment was also present in both medial and lateral compartments, although these results were not always statistically significant. Our study is unable to address whether in subjects with asymptomatic radiographic knee OA, the same relationship exists. However, since cartilage defects and volume have both been shown to correlate strongly with radiographic disease and to be more sensitive to early disease than radiographic changes, we hypothesize that it is likely a continuum that exists in these changes [7, 14]. Because we did not obtain measures of knee alignment (radiographic or clinical) we are unable to examine how this affects the relationships described, which has been shown to be important in cross-sectional studies. However, the absence of these measures is likely to result in non-differential misclassification, which is likely to diminish the magnitude of the results obtained, rather than to result in spuriously positive associations.

These data suggest that BMLs, present in healthy asymptomatic individuals with no history of significant knee pain or trauma, are associated with increased risk of cartilage defect progression and loss of cartilage volume. This suggests that either BMLs or a factor associated with their presence may be important as a target for preventive measures for knee OA.

Rheumatology key messages

- In pain-free knees, a BML increases the tendency of cartilage defects to progress.
- Defects are more likely to progress with increasing size of the BML.

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The Natural History of Bone Marrow Lesions in Community-Based Middle-Aged Women Without Clinical Knee Osteoarthritis

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Objective: Bone marrow lesions (BML) have been implicated in the pathogenesis of osteoarthritis, yet their exact role, etiology, and natural history remain unclear. The aim of this study was to examine the natural history of BML in a healthy population and identify risk factors associated with their persistence and incidence.

Methods: One hundred forty-eight healthy middle-aged women had magnetic resonance imaging performed on their dominant knee at baseline and 2 years later to assess the presence, natural history, and risk factors for persistence and incidence of BML.

Results: Approximately 46% of BML present at baseline completely resolved over 2 years. "Large" BML had the potential to improve, while the majority of "very large" remained stable. In those women with no BML at baseline, approximately 9% developed a BML over 2 years, the majority in the medial compartment. There was a trend toward weight being a risk factor for the development of "very large" BML ($P = 0.08$).

Conclusions: The natural history of BML may be different in healthy persons compared with diseased states. The trend for weight as a risk factor for development of a "very-large" BML suggests there is potential to identify modifiable risk factors for BML in asymptomatic people and warrants further investigation.

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Keywords: ***knee, bone marrow lesions, osteoarthritis, magnetic resonance imaging***

Bone marrow lesions (BML) have been implicated in the pathogenesis of osteoarthritis (OA) (1-3), yet their exact role and etiology remain unclear. They are present in both symptomatic (2-5) and healthy populations without knee pain or any history of significant knee injury (6-8).

Previous studies on the natural history of BML have examined symptomatic populations with established radiographic knee OA (4,5,9-11). In established knee OA, they are associated with pain (2), changes in cartilage metabolism as measured by type II collagen degradation marker CTX-II (4), and increased likelihood of cartilage loss (5,12). There is a paucity of studies examining the natural history of BML in asymptomatic, healthy populations. Moreover, factors associated with the development of incident BML in healthy populations have yet to be examined.

The aim of this study was to examine the natural history of BML in asymptomatic women with no clinical

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knee OA and to identify factors associated with persistence and incidence of BML over 2 years.

METHODS

Participants

Eligible participants were part of a previous cross-sectional study, having been recruited from a database established from the Victorian state electoral roll (Australia) between April 2002 and August 2003 (13). Women were eligible if they were aged 40 to 67 years, had not had a hysterectomy, and had agreed to be recontacted regarding further research studies. Women who had experienced significant knee pain or injury in the last 5 years that necessitated treatment by health professionals, or had required rest for more than 1 day or a history of any arthritis diagnosed by a medical practitioner, were excluded. Subjects with a contraindication to having a magnetic resonance imaging (MRI) scan, such as the presence of a pacemaker, metal sutures, iron filings in the eye, or claustrophobia were also excluded. Of the initial 355 women contacted, 176 were eligible to participate. The study was approved by the Alfred Hospital and Monash University Human Research Ethics Committees, and all participants gave written informed consent.

Data Collection

Weight was measured to the nearest 0.1 kg using a single pair of electronic scales with shoes, socks, and bulky clothing removed. Height was measured to the nearest 0.1 cm using a stadiometer with shoes and socks removed. Body mass index (BMI) (weight/height^2 kg/m^2) was calculated. At follow-up, we assessed pain using the Western Ontario and McMaster Universities Osteoarthritis Index pain dimension (5 items using visual analog scores, where higher scores indicate worse status) (14), with a possible range of scores 0 to 500. A score of $\geq$100 of 500 (20%) was considered representing development of incident knee pain.

MRI and the Measurement of BML

An MRI of the dominant knee of each subject (defined as the lower limb from which the subject stepped off from when initiating gait) was performed between October 2003 and August 2004 and approximately 2 years later. Knees were imaged in the sagittal plane on a 1.5-T whole-body magnetic resonance unit (Philips) using a commercial transmit-receive extremity coil. The following sequence and parameters were used: a T1-weighted fat-suppressed 3D gradient recall acquisition in the steady state; flip angle 55°; repetition time 58 milliseconds; echo time 12 milliseconds; field of view 16 cm; 60 partitions; 512 × 196 matrix; 1 acquisition time 11 minutes 56 seconds. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31 × 0.83 mm (512 × 196 pixels). In addition, coronal images were obtained using a T2-weighted fat-saturated fast spin-echo acquisition, repetition time 2200 milliseconds, echo time 20/80 ms, with a slice thickness of 3 mm, a 0.3 interslice gap, 1 excitation, a field of view of 11 to 12 cm, and a matrix of 256 × 128 pixels was also obtained.

Assessment of BML

BML were defined as areas of increased signal intensity adjacent to subcortical bone present in either the medial or lateral distal femur or the proximal tibia (2). These were differentiated from cysts. Two trained observers, who were blinded to patient characteristics, as well as sequence of images, together assessed the presence of lesions for each subject. The presence or absence of a BML was determined. A lesion was defined as "large" if it appeared on 2 or more adjacent slices and encompassed at least one-quarter of the width of the tibial or femoral cartilage being examined from coronal images (2) (Fig. 1). This is comparable to the previously described "grade 2" BML by Felson (2). Lesions were further classified as "very large" if they appeared on 3 or more slices (2) (Fig. 2). This is comparable to the previously described "grade 3" by Felson (2). Characterization of "large" and "very-large" BML was mutually exclusive. If a person had more than 1 BML underlying a cartilage plate, the BML of the highest grade was used for analysis. Subjects were classified as developing an incident BML if they had no BML in any compartment at baseline but developed BML during the course of observation. The reproducibility for determination of the BML was assessed using 60 randomly selected knee MRI (κ value 0.88; $P < 0.001$).

Statistical Analysis

The site of prevalent and incident BML, as well as persistent BML, was tabulated. The relationship between po-

Figure 1 Medial femoral "large" BML.

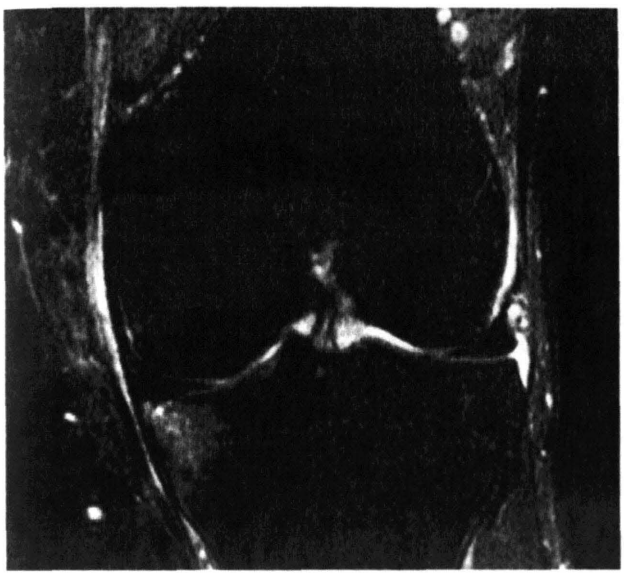

Figure 2 Medial tibial "very-large" BML.

tential risk factors for persistent and incident BML was assessed using binary logistic regression. Potential confounders including age and BMI were included in the multiple regression equation. The relationship between subjects who had BML increase in size with the development of incident knee pain, and the relationship between subjects who developed incident BML with development of incident knee pain was assessed using the χ^2 test for comparison of proportions. A P value of less than 0.05 (2-tailed) was regarded as statistically significant. Analyses were performed using the SPSS statistical package (standard version 14.0, SPSS, Chicago, IL).

RESULTS

One hundred forty-eight women of the 176 (84%) completed follow-up. Twenty-eight women did not undergo a second MRI because of death ($n = 1$), migration ($n = 1$), knee injury ($n = 4$), surgery ($n = 1$), withdrawal of consent ($n = 3$), and being unable to be contacted ($n = 18$). Apart from being younger (mean age (±SD) was 49.5 (±6.3) years, $P = 0.02$ for difference), the baseline characteristics of these women were not significantly different to those who completed the longitudinal component of the study and are presented in Table 1. Over the 2-year study period, only 5 of 148 subjects (3%) developed incident knee pain (Western Ontario and McMaster Universities Osteoarthritis Index score of ≥100 of 500).

Natural History of Prevalent BML

The natural history of subjects with prevalent BML is presented in Table 2. Twenty-two of the 148 (15%) subjects had a BML at baseline; 10 (46%) subjects had BML that completely resolved. In the 11 subjects with a BML in the medial compartment, 6 BML resolved. In the 11 subjects with BML in the lateral compartment, 4 resolved.

At baseline, 14 subjects had a "large" BML and 8 subjects had a "very-large" BML. Of the 14 subjects with "large" BML, 2 remained stable, 3 increased in size, and 9 completely resolved. Seven of the 8 subjects who had a "very-large" BML at baseline remained stable. Using logistic and multiple logistic regression models, no significant relationship between age, weight, or BMI on persistence of BML of any size was observed (data not shown). Of the 3 subjects who had a "large" BML increase in size, none developed incident knee pain, and there was no significant difference in the development of incident knee pain between those subjects who had a "large" BML increase in size and those subjects who had a "large" BML remain stable/completely resolve ($P = 0.59$).

Incidence of BML

The number of subjects who developed an incident BML is presented in Table 3. One hundred twenty-six participants had no BML at baseline. Over 2 years, 11 (9%) of these 126 subjects developed a new BML. Eight subjects developed a BML in the medial compartment, of which 6 were characterized as "large" and 2 were characterized as "very large" (1 subject with a "very-large" incident BML also developed a "large" BML). Three subjects developed a BML in the lateral compartment, of which 2 were characterized as "large" and 1 was characterized as "very large."

Using logistic and multiple logistic regression models, no significant relationship between age, weight, or BMI on development of any incident or "large" BML was observed (data not shown). However, there was a suggestion that weight is associated with development of "very-large" BML after adjustment for potential confounders including age and height [OR: 1.5 (95% CI 0.96, 2.2)] ($P = 0.08$). Of the 11 subjects who developed an incident BML, none developed incident knee pain, and there was

Table 1 Characteristics of Study Population at Baseline Comparing Those Who Completed 2-year Follow-Up and Those Lost to Follow-Up

	Completers ($n = 148$)	Lost to Study ($n = 28$)	P Value
Age (years)	52.8 (6.6)	49.5 (6.3)	0.02
Height (cm)	163.7 (6.5)	165.8 (6.3)	0.11
Weight (kg)	72.9 (14.4)	71.6 (12.3)	0.65
BMI (kg/m^2)	27.3 (5.7)	26.0 (4.2)	0.26
Presence of any knee BML[a]	22 (15%)	5 (18%)	0.69
Presence of "large" BML[a]	14 (10%)	4 (14%)	0.52
Presence of "very large" BML[a]	8 (5%)	1 (4%)	0.69

Values are reported as mean (SD) at baseline unless otherwise stated. t-tests were used for comparison of means.
[a]χ^2 tests were used for comparison of proportions.

Table 2 Natural History of the 22 Subjects with Prevalent BML at Baseline

	Number of Subjects with BML at Baseline	Number (%) of Subjects in Whom BML Had Resolved at Follow-Up
Any medial BML	11	6 (55)
Any lateral BML	11	4 (36)
Large BML	14	9 (64)
Very large BML	8	1 (13)

no significant difference in the development of incident knee pain between those subjects who developed an incident BML and those who did not ($P = 0.53$).

DISCUSSION

In this study of asymptomatic women without clinical knee OA, we found that approximately 46% of BML present at baseline completely resolved over 2 years and approximately 9% of women who were free of a BML developed a BML. Of the 14 subjects with a "large" BML present at baseline, 2 remained stable, 3 increased in size, and 9 completely resolved. However, the majority (88%) of "very-large" BML at baseline remained stable over 2 years. The majority of subjects developed their new BML in the medial compartment and there was a trend toward weight being a risk factor for development of an incident "very-large" BML.

The natural history of BML has been reported previously in populations mixed in regards to radiographic knee OA and the presence of pain (5,10). In predominantly obese subjects with symptomatic radiographic OA, BML were unlikely to resolve with 99% of BML either persisting or increasing in size over 15 or 30 months (5). In a population mixed in regards to radiographic OA and pain, only 10% of BML disappeared over 2 years (10). Moreover, in a population with chronic knee pain mixed in regards to radiographic OA, 37% of BML increased in size, 19% decreased in size, and 22% disappeared over 2 years (11). Data also suggest that BML incidence in those with symptomatic radiographic OA was approximately 20% over approximately 2 years (5).

We have shown that in asymptomatic women without clinical knee OA, the likelihood of complete resolution of BML is higher than previously reported in symptomatic and OA populations (5,10,11). "Large" BML had the potential to resolve, while the majority of "very large" remained stable and did not improve. Previous studies have suggested that persistence or worsening of BML may be explained by a combination of increased loading compounded by obesity and systemic (15) or dietary factors (16). We were unable to identify risk factors for persistence or worsening of BML, likely due to insufficient power. Nevertheless, the results of this study suggest that in asymptomatic populations there is potential for improvement and that the natural history of BML may be a function of the size of the initial BML. Moreover "large" BML may represent a more useful target for future intervention studies.

Table 3 The Number of Subjects Who Developed an Incident BML Over 2 Years in Subjects Who Were Free of BML at Baseline ($n = 126$)

	Number (%) of Subjects in Whom an Incident BML Developed at Follow-Up
Medial	
Large	6 (5)
Very large	2 (2)
Lateral	
Large	2 (2)
Very large	1 (1)

Our finding that BML developed in approximately 9% of people over 2 years is lower than in symptomatic populations and suggests that not only are BML more common in OA, but the rate of BML development in healthy populations may also be less than those with knee pain or radiographic OA (5). There was a trend for increasing weight to be associated with the development of a "very-large" BML, and the majority of these incident BML developed within the medial compartment. This may be related to increased loading and is consistent with previous research that suggests the natural history of BML may be mediated by biomechanical factors such as static knee alignment (5). These results suggest that development of BML may be attenuated by modifiable risk factors such as obesity and warrants further investigation.

A limitation of our study is that we examined only asymptomatic women; these results may not be generalizable to men or to symptomatic populations. In addition, radiographs were not available; thus, it is possible that some subjects may have early signs of subclinical OA. Nevertheless, we excluded those subjects who had experienced significant knee pain or injury in the last 5 years or a history of any arthritis diagnosed by a medical practitioner. Given the small number of persistent and incident BML, our power to detect associations with potential risk factors was limited. In addition, given that only 5 subjects developed incident knee pain over the study period, our power to detect a relationship between subjects who had BML increase in size, and subjects who developed incident BML with development of knee pain was low, and larger studies of longer duration will be required. Moreover, future studies may benefit from examination of

other biomechanical risk factors for BML, such as static knee alignment.

In asymptomatic women with no clinical knee OA, the incidence of BML is lower than previously reported in symptomatic subjects and prevalent BML were more likely to resolve. This suggests that BML may represent a potential therapeutic target in the prevention of OA and requires further investigation.

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Imaging of knee osteoarthritis

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New imaging modalities are broadening the possibilities in osteoarthritis (OA) research, and are offering new insights to help better understand the pathogenesis of this disease. Although knee radiographs are widely employed in epidemiological and clinical studies to assess structural pathology, joint radiographs provide limited outcome measures in knee OA, and other more valid, reliable and sensitive imaging modalities are now available. In particular, magnetic resonance imaging can directly visualize articular cartilage and other joint structures, such as bone and soft tissue, that are now recognized as part of the disease process. This chapter will examine imaging modalities in the assessment of knee OA, and the impact of these on our understanding of the pathogenesis of this disease.

Key words: osteoarthritis; radiology; magnetic resonance imaging; radiograph.

Osteoarthritis (OA), the most common form of arthritis, is the third leading cause of disease burden, measured as disability-adjusted life years, in the developed world. This is predicted to increase over the coming decades.[1] The disease commonly affects weight-

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bearing joints, such as the knee, and the major clinical features of OA are pain and stiffness, leading to a decline in physical functioning. Radiological OA is estimated to be prevalent in 30% of people over 65 years of age.[2] The disease is radiographically characterized by joint space narrowing (JSN), osteophytes, subchondral cysts and subchondral sclerosis. Despite these recognized structural changes, the aetiology of this condition remains unclear. In part, this may be due to the lack of a sensitive tool to assess OA severity and progression.

Although the current gold standard for assessing OA in clinical and epidemiological settings is the radiograph, there are significant limitations associated with this approach. Expert consensus has suggested that in trials of disease-modifying therapy in knee OA, a measure of cartilage should be used.[3] Ideally, this should be a valid (i.e. actually measures cartilage) and reliable (i.e. gives the same result when repeated under identical circumstances) measure of cartilage that is also sensitive to change (i.e. has the ability to show clinically important anatomical change). The joint radiograph does not satisfy all these criteria: it provides an indirect, surrogate measure of articular cartilage via an assessment of the radiological joint space width. Moreover, joint radiographs are limited by their two-dimensional assessment of bony features, and cannot identify three-dimensional changes in intra- and extra-articular structures, such as bone size and cartilage defects, that are now recognized as part of the disease process.

More modern imaging modalities, particularly magnetic resonance imaging (MRI) which enables a three-dimensional assessment of the entire joint, have offered a quantitative alternative to radiographs for examining the pathogenesis of OA.[4–6] In epidemiological studies that have utilized MRI to examine joint morphology, there is emerging evidence that there may be a continuum from the normal knee, through to the preclinical, asymptomatic state, and finally the development of clinical knee OA.[7–10] However, the utility of MRI and other imaging modalities in the outcome assessment of knee OA has been restricted to research settings alone. The future challenge remains in the clinical applicability of novel imaging modalities to provide early identification of people with or at risk of knee OA, so that appropriate interventions can be identified and instituted to reduce disease incidence. This chapter will examine the use of different imaging modalities in the assessment of knee OA, and the subsequent advances related to the pathogenesis of the disease that have been made from such novel modalities (Figure 1).

RADIOGRAPHIC ASSESSMENT OF KNEE OSTEOARTHRITIS

Radiological grading of knee osteoarthritis

Radiographs have been widely used as an outcome measure in the disease. Kellgren and Lawrence first described a grading system in 1957 that was later adopted by the World Health Organization in 1961 as the standard measure for assessing radiographic OA.[11] Subsequently, methods which grade the individual features of OA, such as osteophytes, JSN and subchondral sclerosis, have been used.[12] However, there are several limitations associated with both of these grading systems.

Firstly, these grading systems predominantly employ ordinal measures, with only a limited number of categories. These yield relatively crude and insensitive measures of disease progression. Moreover, the role of osteophytes, which are central to grading systems, is unclear. For instance, although related to the presence of pain, osteophytes are not related to the severity of pain.[13] Thus, grading systems may lack complete content validity.[14] Furthermore, osteophytes have not been shown to be associated with disease progression.[15] Kellgren and Lawrence's grading system

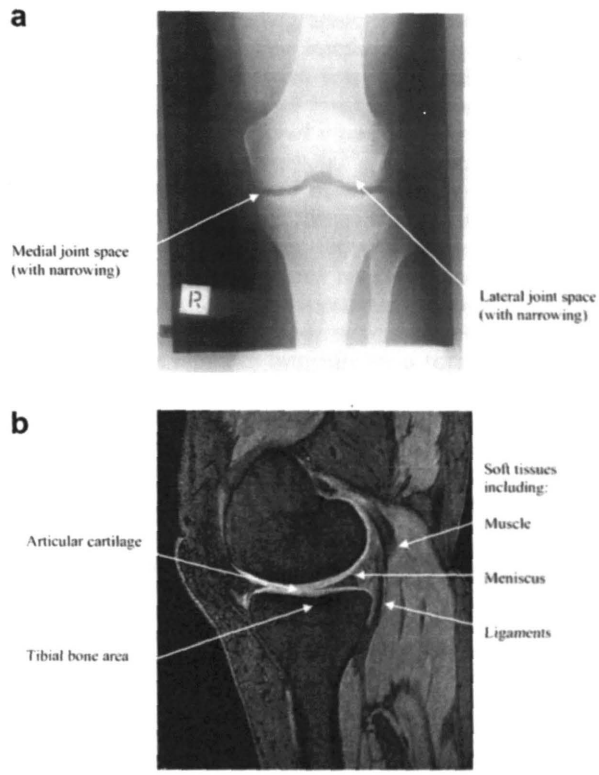

Figure 1. Comparison of the information obtained from (a) knee X-ray and (b) T1-weighted magnetic resonance imaging (sagittal).

relies heavily on the presence of osteophytes for the identification of knee OA. Studies examining individual radiographic features of OA have also tended to predominantly report results related to the assessment of osteophytes since measurements of osteophytes tend to yield better reproducibility than measurements of JSN.[16] This focus on the presence of osteophytes in epidemiological studies may therefore have underemphasized findings related to JSN.[17–19] For instance, it was concluded that variables such as physical activity may be detrimental to the knee joint given that a higher prevalence of osteophytes was identified in the knees of persons exercising more vigorously.[16] In the same study, however, the severity of JSN was lower among people exercising more vigorously compared with less active controls. Taken together, these findings may actually infer that physical activity benefits the knee joint by retarding JSN, and the osteophytes may be the result of musculoskeletal traction forces.

Measurement of joint space width/narrowing

JSN, a continuous measure, has been employed as the outcome in studies of disease progression in OA.[20] Underlying use of JSN is the assumption that longitudinal reduction in joint space is a valid measure of a reduction in articular cartilage volume. This is

not necessarily true, since the radiographic joint space is comprised of structures other than articular cartilage.[21,22] For instance, mensical extrusion has been shown to account for much early joint space loss.[18] Reliability issues related to JSN are also problematic, primarily because of measurement error related to positioning of the knee. For example, the conventional anteroposterior knee radiograph is highly observer dependent when determining JSN,[23] and measurement error may relate to both the positioning of the knee, and its alignment with respect to the radiograph and source of radiation.[3,24,25] Various methods to improve reproducibility have been proposed and validated over the short term.[26,27] However, when one of these, the metatarsophalangeal view, was tested for sensitivity to change, the results were not biologically plausible.[28] Moreover, JSN is relatively insensitive to change and few studies have demonstrated significant change over short-term periods.[29,30] For instance, radiological assessment at 6-month intervals for a total period of 2 years was unable to distinguish significant changes in JSN among people with knee OA, although a significant loss of articular cartilage volume was detected.[29] Although use of the more technically challenging Lyon schuss position, which employs a semi-flexed anteroposterior radiograph with fluoroscopy to align the tibial plateaux, demonstrated sensitivity to change, this has not been validated against a direct measure of cartilage.[30]

IDEAL IMAGING MODALITY FOR STRUCTURAL SEVERITY OF OSTEOARTHRITIS

The ideal imaging modality for the progression and assessment of OA would provide data pertaining to all joint structures, including a direct measure of both cartilage and bone, as well as other intra- and extra-articular structures in three dimensions. The ideal measure would be non-invasive, readily available, cheap, valid, reliable, relate to clinical outcomes and be sensitive to change without exposing a subject to unnecessary ionizing radiation. MRI promises to fulfil many of these criteria.

MAGNETIC RESONANCE IMAGING

In the setting of knee OA, MRI studies initially focused on the assessment of articular cartilage as the main outcome measure, as an extension of the previous approach using radiological grading, particularly JSN, in clinical and epidemiological studies. MRI has been seen as a potentially major advance since it enables the direct assessment of cartilage, rather than the indirect approach allowed by use of radiology. Subsequent methods have been developed to assess many properties of articular cartilage using MRI, including thickness and volume, as well as detecting and classifying surface irregularities, known as cartilage defects, and identifying other structural changes in the knee.

Measures of cartilage

Semiquantitative measurement of articular cartilage in knee osteoarthritis

There have been attempts to develop sensitive grading systems to assess cartilage signal and morphology.[5,31] These methods have divided the articular cartilage into regions and graded the surface according to depth and area of lesions, as an extension from arthroscopic measures of cartilage defect severity. Although some of these methods have been

shown to be sensitive to change,[32] these outcomes have not been validated against clinical endpoints (e.g. symptoms, function, joint replacement).[31]

Measurement of articular cartilage thickness in knee osteoarthritis

Measuring articular cartilage thickness via MRI has been examined as a quantitative alternative to the radiological assessment of the severity of OA, as well as to identify risk for disease onset and progression.[33,34] Previous work has generally employed T1-weighted fat suppression images to assess articular cartilage properties.[6,33,35] However, several limitations have been identified when adopting cartilage thickness as a means of assessing knee OA. In particular, diurnal variability in articular cartilage thickness, but not volume, has been demonstrated.[34] Additionally, longitudinal studies examining change in cartilage thickness may be limited by the difficulty in reselecting identical section locations at follow-up assessment.[36]

Articular cartilage volume

Validity, reliability and sensitivity to change. MRI directly images articular cartilage and therefore demonstrates face validity. Content validity (i.e. the degree to which the instrument represents a specified universe) has also been shown, with a correlation of 0.98 observed between cartilage volume obtained from MRI assessment and cartilage volume measured from surgical and post-mortem specimens.[6,35,37,38] Moreover, the assessment of articular cartilage volume appears to be a valid indicator of particular clinical outcomes, including change in symptoms and the likelihood of progression to knee replacement surgery.[35,39–42] In particular, individuals who were in the top tertile of rate of cartilage loss over 2 years were shown to have a seven-fold increased risk of progressing to a knee replacement within 4 years compared with those in the lowest tertile.[39]

Assessing knee articular cartilage volume from MRI is reliable,[43] with low coefficients of variation, as a measure of intra-observer variability, reported for healthy (2.4–2.6%) and arthritic (2.9–3.2%) subjects in both the medial and lateral tibiofemoral compartment, as well as the patellofemoral compartment.[44] High interobserver reliability has also been demonstrated, with coefficients ranging from 0.97 to 0.99 for both healthy and arthritic joints.[44]

MRI is also a sensitive measure of change in cartilage volume. The finding that 11–13% of cartilage volume is lost before the first changes of radiographic JSN can be detected[9] provides the strongest evidence yet that measurement of MRI-derived cartilage volume is a far more sensitive measure of early cartilage loss (i.e. preclinical disease) than radiographic JSN. This is supported by a report of significant loss in cartilage volume documented at 6-monthly intervals for a period of 2 years in people with knee OA, despite no correlation seen between cartilage volume loss and radiographic changes.[29] Sensitivity to change has also been demonstrated in people without established knee OA.[45] Other studies have demonstrated sensitivity to change in different contexts, such as in spinal cord injury patients[46] and even after 7 weeks of partial immobilization for ankle fracture.[47]

Measurement of articular cartilage defects

While a reduction in cartilage volume is a recognized feature of degenerative change, other earlier cartilage lesions, often referred to as cartilage defects, are also apparent

prior to radiographic change.[48] Cartilage defects are irregularities on the surface of the usually smooth articular cartilage that can be detected and assessed via MRI. Histologically, these are highly correlated with the Mankin scale for grading cartilage.[49] Although the natural history of cartilage defects is speculative, they appear to be present in people without pain and radiological OA, as well as in people with well-established, painful radiological OA.[7,48,50–53]

Among asymptomatic individuals, there is growing evidence that the presence of cartilage defects may represent early OA.[54,55] The presence of cartilage defects in asymptomatic subjects has been associated with a reduction in cartilage volume.[7,50] Over 2 years in an asymptomatic population, an increase in knee cartilage defect score (change of ≥ 1) was associated with higher rates of knee cartilage volume loss, whereas a decrease in the knee cartilage defect score (change of ≤ 1) was associated with a relative increase in knee cartilage volume.[54,55] In another healthy population, the presence of asymptomatic, non-full-thickness medial tibiofemoral cartilage defects identified healthy individuals most likely to lose knee cartilage over 2 years in the absence of radiographic knee OA.[48] Whether asymptomatic individuals with knee cartilage defects are at increased risk of developing clinical knee OA has not yet been examined. Nevertheless, in people with knee OA, cartilage defects predict more rapid cartilage loss and disease progression.[7] Moreover, the severity of cartilage defects is also associated with markers of disease progression, including the risk of total knee joint replacement,[56] as well as pain and disability scores.[51–53]

In both healthy and osteoarthritic states, more severe cartilage defects exhibit a 'dose–response' association with cartilage volume and its loss, and are associated with radiographic severity of knee OA.[7,48,54–56] These data, derived from MRI examination of cartilage defects, therefore demonstrate the potential for a continuum to exist which extends from the presence of asymptomatic cartilage defects to the endpoint of clinical knee OA where knee joint replacement is indicated. Nevertheless, it must be recognized that the presence of cartilage defects does not mandate the onset or progression of knee OA in all subjects. Which factors instigate and cause progression of cartilage defects has yet to be fully characterized.

Measurement of glycosaminoglycan content of articular cartilage

While MRI can readily identify morphological abnormalities, recent developments have enabled the extension of standard MRI techniques to provide a measure of the biochemical composition and functional properties of joint structures. Delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) is based on the principle that both gadolinium, an MRI contrast agent, and glycosaminoglycan (GAG), the major proteoglycan of cartilage, are negatively charged. When allowed to penetrate cartilage, gadolinium is subsequently excluded from regions that are rich in GAG, and an index of the GAG concentration is made. Several studies that have utilized dGEMRIC have observed differences in the GAG index between knee compartments[57,58] and can identify GAG loss in patients with early OA,[58,59] suggesting a potential role for dGEMRIC in helping to better understand the natural history of OA and the effects of interventions. For instance, one study demonstrated differences in GAG concentrations between exercising and sedentary volunteers based on dGEMRIC assessment,[60] which supports recent findings obtained from standard MRI studies demonstrating a beneficial effect conferred by physical activity on knee cartilage.[61] In addition to enabling mapping of the biochemical composition of cartilage, dGEMRIC may also offer a non-invasive assessment of mechanical cartilage properties since high correlations between dGEMRIC and site-matched measures of cartilage stiffness are seen.[62]

Structures other than articular cartilage are affected in osteoarthritis

While cartilage has been the predominant focus of studies examining knee OA, other joint structures are also involved in the pathogenesis of the disease. MRI examines other intra- and extra-articular structures. The assessment of articular cartilage volume in isolation, even after adjustment for bone size, is unlikely to be the best available means of determining the severity of OA: use of a combination of measures of multiple joint structures associated with disease (e.g. bone, meniscus, etc.) may help to better assess the severity of knee OA.

Bone marrow lesions

The role of bone marrow lesions (BMLs) in the pathogenesis of knee OA has generated interest recently. BMLs are correlated with increased bony uptake on bone scintigraphy[63,64] and histologically demonstrate features consistent with ongoing bone trauma, including abnormal bone formation with excessive fibrosis, extensive bony remodelling with reversal lines and areas of osteonecrosis.[65]

BMLs have been shown to be prevalent in approximately 13% of healthy middle-aged subjects with no clinical knee OA.[66] In these healthy subjects, BMLs were positively associated with tibial bone area and were associated with an increased risk of the presence of cartilage defects, without being associated with a reduction in cartilage volume.[66,67] In people with knee OA, BMLs are associated with the radiographic progression of compartment-specific JSN, although some of this progression may have been attenuated by frontal plane knee alignment.[68] Similarly, knee compartments with a higher baseline BML score also had greater cartilage loss at follow-up.[32] However, enlarging or new BMLs mainly occurred in malaligned limbs, on the side of the malalignment (e.g. new medial BMLs in varus-aligned knees), and the association between BML change and medial tibiofemoral cartilage loss was not significant after adjusting for alignment.[32] Taken together, these findings suggest that BMLs may be detrimental to the health of the knee joint, and that biomechanical factors may be just one of many factors underlying their pathogenesis.

Metaphyseal bone expansion

MRI studies have shown that people with knee OA have a larger bone surface area at the tibial plateau than healthy controls, and that the rate of bone expansion is greater in osteoarthritis than in healthy subjects.[69,70] Radiographically, for each increase in grade of JSN in a compartment, tibial plateau bone area was also shown to be increased.[10] It has been speculated that bone expansion may reflect the state of articular cartilage health at the knee.[71] Recently, an MRI study inferred that an increased surface area of the tibia plateau was a major determinant in mediating reduced knee cartilage volume among people without clinical knee OA.[72] This suggests that bone expansion may be important and an independent factor initiating cartilage change. Moreover, it was recently shown that a larger bone area at baseline was predictive of an increase in the severity of knee cartilage defects over 2 years.[48,73]

Other changes observed within the joint in knee osteoarthritis

Meniscal tears and extrusions

Meniscal tears have been reported to be prevalent among 91% of people with symptomatic knee OA, and a high percentage (76%) of asymptomatic age-matched

controls.[74] Meniscectomy is known to increase the rates of symptomatic and radiographic OA,[75] and meniscal tears and partial meniscectomy lead to increased rates of cartilage loss.[31,76] In a largely non-OA cohort, meniscal tears were associated with cartilage defect development and progression, loss of cartilage volume and alteration in bone size.[77] Moreover, the risk factors for meniscal tears were shown to share similar risk factors as for knee OA, including age, body mass index, female gender and genetics.[77]

Similarly, another study demonstrated that baseline meniscal extrusions were associated with increased knee cartilage loss over 2 years in a non-OA cohort.[72] This association appeared to be predominantly mediated by subchondral bone changes, suggesting that extrusion represents one pathway between bone expansion and cartilage loss. Taken together, these data suggest that meniscal lesions may be a risk factor for knee cartilage damage and articular structural changes.

Ligamentous damage

Appreciation of the role of knee ligaments in the natural history of knee OA has largely been derived from studies of anterior cruciate ligament (ACL) rupture. For instance, 50–70% of patients with complete ACL rupture and associated injuries have radiographic changes consistent with OA after 15–20 years.[78] In painful knee OA, complete ACL rupture was seen in 23% vs 3% of age-matched non-painful knees without recollection of rupture, indicating that ACL rupture is more common in symptomatic knee OA.[79] Moreover, in another recent study, central BMLs abutting the ACL were highly prevalent and strongly related to ACL pathology in people with knee OA.[80] In another study examining joint laxity, greater varus-valgus laxity was demonstrable in the uninvolved knees of patients with unilateral knee OA compared with older control knees, supporting the concept that some portion of the increased laxity of OA may predate disease.[81]

Synovial changes

Chronic inflammatory changes with production of pro-inflammatory cytokines are a feature of synovial membranes from patients with early knee OA, with the most severe changes seen in patients at the time of joint replacement surgery.[82] MRI studies have substantiated that the degree of synovial thickening in people with knee OA correlates with qualitative macroscopic analysis and microscopic features (synovial lining cells, surface fibrin deposition, fibrosis, oedema, congestion and infiltration).[83,84] Synovial thickening in people with knee OA also appears to be a determinant of knee pain, even when the radiological grade of OA is taken into account.[85]

OTHER IMAGING MODALITIES IN KNEE OSTEOARTHRITIS

Although MRI has the advantage of direct visualization of joint structure, other modalities, such as ultrasound and nuclear scans (including single photon emission computed tomography), have also been used to provide complementary information. Nevertheless, the majority of the limited research studies available have examined in-vitro animal models and there is a paucity of studies using novel imaging modalities to examine human subjects in vivo, despite their use in the clinical assessment of arthropathies.

Ultrasound

Clinically, ultrasound is widely employed to provide imaging guidance for procedures such as intra-articular injection and biopsy for both the investigation and treatment of joint arthropathies. The utility of ultrasound to provide real-time clinical information about the state of articular health has not been widely examined in humans. This may be due to the inaccessibility resulting from small acoustic windows obtained from in-vivo specimens, which therefore limits the evaluation of intra-articular structures such as menisci, cruciate ligaments and articular cartilage surfaces.[86] However, in-vitro studies of bovine articular cartilage have shown that ultrasound may be used to detect collagen disruption and increased roughness in articular cartilage after specimens were subject to mechanical or enzymatic degradation.[87] In another study examining bovine cartilage, ultrasound provided a sensitive measure to differentiate samples of intact cartilage from early and more advanced cartilage degeneration, determined histologically using the Mankin score.[88] Such findings suggest that ultrasound examination may be helpful in detecting early OA by imaging articular and peri-articular cartilage and soft tissues, in the absence of clinical symptoms.[89]

Nuclear imaging

Nuclear imaging may offer further insight into the early disease processes occurring in OA. For instance, a study that utilized positron emission tomography demonstrated increased 2-deoxy-20[18F]fluoro-D-glucose uptake in joints of patients with OA.[90] However, most nuclear studies examining OA have utilized bone scintigraphy. Early bone scintigraphy work demonstrated that the activity of subchondral bone was able to predict subsequent loss of joint space in people with established knee OA.[91] A later study comparing bone scintigraphy, MRI and radiographs in people with knee OA demonstrated a significant association between increased bone uptake and MRI-detected subchondral lesions.[63] This adds further weight to the notion that changes in subchondral bone may initiate cartilage degeneration. In another study, serum concentrations of cartilage oligomeric matrix protein and bone sialoprotein were significantly higher in individuals with than without bone scan abnormalities,[92] suggesting that serum markers may be important in detecting people with or at risk of degenerative change. Despite the paucity of nuclear studies examining OA, the role of this imaging modality appears to offer another potential assessment tool to help better understand the natural history of the disease, particularly in relation to bone changes which may precede articular cartilage destruction.[93]

CONCLUSION

The use of improved non-invasive techniques to visualize the joint have demonstrated that OA is a disease which affects the whole joint, and it is possible to examine structural changes as a continuum from the normal through to the diseased knee joint. Future studies that exploit this continuum are likely to improve our understanding of the natural history of OA, and to identify preclinical individuals at risk of developing knee OA. It is becoming increasingly evident that knee OA may be the clinical manifestation of a multitude of changes in joint structure, which have their origins in the apparently healthy, asymptomatic state. These early changes may provide novel targets for the prevention and treatment of knee OA.

Practice points

- new imaging modalities are broadening the possibilities in osteoarthritis research, and are offering new insights to help better understand the pathogenesis of this disease
- joint radiographs provide limited outcome measures in knee OA, and other more valid, reliable and sensitive imaging modalities are now available
- in epidemiological studies that have utilized MRI to examine joint morphology, there is emerging evidence that there may be a continuum from the normal knee, through to the preclinical, asymptomatic state, and finally the development of clinical knee OA
- the future challenge remains in the clinical applicability of novel imaging modalities to provide early identification of people with or at risk of knee OA so that appropriate interventions can be identified and instituted to reduce disease incidence

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Bone marrow lesions predict increase in knee cartilage defects and loss of cartilage volume in middle-aged women without knee pain over 2 years

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ABSTRACT

Objective: Bone marrow lesions (BML) are important in established knee osteoarthritis, predicting pain and progression of disease. Whether BML are also associated with longitudinal changes in knee structure in an asymptomatic population is unknown.

Methods: 148 healthy pain-free women in middle age with no history of knee injury or clinical knee osteoarthritis who had a magnetic resonance imaging (MRI) scan performed on their dominant knee at baseline, had another MRI 2 years later to assess whether having a BML present at baseline affected change in tibiofemoral cartilage defects and tibial cartilage volume.

Results: BML were present in 14.9% of women at baseline. The risk of progression of total tibiofemoral cartilage defects was significantly higher when a very large BML was present (odds ratio 5.55, 95% CI 1.04 to 29.6) compared with when no BML was present, after adjusting for potential confounders. In the lateral compartment, the rate of cartilage volume loss was significantly greater when a BML was present after adjusting for confounders (regression coefficient 39.2 mm^3, 95% CI 11.1 to 67.2, $p = 0.007$).

Conclusions: In healthy women without pain at baseline, large BML were associated with both progression of cartilage defects in the whole tibiofemoral joint and more rapid lateral tibial cartilage loss. These data suggest that the relationship between BML and knee cartilage in healthy women is similar to that described in established osteoarthritis. It is possible that BML may predict an increased risk of knee osteoarthritis and facilitate the identification of novel interventions to prevent disease.

Osteoarthritis is a complex disease, involving all the tissues of the affected joint. Whereas the tissue of origin of osteoarthritis is controversial, subchondral bone appears important in early disease.[1] Both human and animal studies have suggested that bone changes may precede cartilage damage.[2 3] Once osteoarthritis is established, increased subchondral bone metabolism has been linked to disease progression.[4] This relationship may be mediated by local cytokine release, as in established osteoarthritis bone explants are able to affect cartilage metabolism.[5]

Bone marrow lesions (BML), visible using magnetic resonance imaging (MRI), have been recognised as a feature of knee osteoarthritis.[6 7] In established knee osteoarthritis, these are associated with pain and an increased likelihood of cartilage loss.[6–8] They are present in approximately 10% of healthy middle-aged individuals without knee pain or a history of significant knee injury, and have been attributed to biomechanical factors or systemic factors such as diet.[7 9–12] Two cross-sectional studies in different populations suggested that BML are associated with age, height and body mass index (BMI), all of which are risk factors for knee osteoarthritis.[10 11] BML have also been consistently associated with an increased prevalence and severity of cartilage defects and metaphyseal expansion, both characteristics of the increasing severity of knee osteoarthritis.[10 11] Although longitudinal studies in osteoarthritis suggest that BML are linked with increased cartilage loss, there are no similar studies in asymptomatic individuals. Therefore, whether BML predict structural change, which in turn may progress to knee osteoarthritis in a pain-free population, is unknown.

The aim of this study was thus to examine, in a population of women in midlife, with no knee pain and thus no clinical knee osteoarthritis, the relationship between BML and changes in knee cartilage defects and volume over 2 years.

METHODS

This was a prospective cohort study in which eligible participants aged 40–67 years were recruited to examine factors affecting knee cartilage from a longitudinal cohort study of normative hormone levels in well women.[13] These women were initially recruited from a database established from the electoral roll in Victoria, Australia, between April 2002 and August 2003.[13] Participants underwent a baseline MRI scan on their dominant knee between October 2003 and August 2004 and a follow-up MRI scan on the same knee approximately 2 years later.[14] Women who had experienced significant knee pain (ie, pain requiring any intervention by a health professional, medication or necessitating non-weight-bearing therapy) or a knee injury in the past 5 years that necessitated treatment or required rest for more than one day, or who had a contraindication to undergoing MRI, or who were unlikely to be available to complete the full 2-year study were excluded.[14] Knee radiographs were not obtained. The study was approved by the Alfred Hospital and Monash University Human Research Ethics Committees. All participants gave written informed consent.

Anthropometric data

Each participant's height and weight was measured at the time of the original study (2002–3). BMI was calculated from these data (weight (kg)/height^2 (m^2)).

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Table 1 Characteristics of women with and without BML at baseline

	BML at baseline N = 22	No BML at baseline N = 126	p Value*
Age, years	53.1 (6.3)	52.8 (6.7)	0.87
Height, cm	165.4 (6.3)	163.4 (6.5)	0.17
Weight, kg	78.4 (18.9)	71.9 (13.3)	0.14
BMI, kg/m^2	28.7 (6.9)	27.0 (5.4)	0.30
Baseline tibial cartilage volume, mm^3			
Medial	1611 (347)	1556 (291)	0.43
Lateral	1811 (342)	1788 (351)	0.78
Total	3422 (574)	3345 (569)	0.56
Baseline tibial plateau area, cm^2			
Medial	1902 (192)	1844 (172)	0.15
Lateral	1246 (143)	1180 (129)	0.03
Total	3149 (309)	3024 (250)	0.04
Baseline tibiofemoral cartilage defect score (median (range))†			
Medial	3 (1–7)	2 (0–5)	0.001
Lateral	3 (1–6)	2 (0–7)	<0.001
Total	6 (4–11)	4 (1–10)	<0.001

Values are expressed as means (standard deviation), except where indicated. *Unpaired t-test for difference, except where indicated. †Expressed as median (range) difference examined using Somer's d-test. BMI, body mass index; BML, bone marrow lesion.

MRI and the measurement of cartilage volume, defects, bone area and BML

MRI

An MRI of the dominant knee (defined as the lower limb from which the subject stepped off when initiating gait) was performed between October 2003 and August 2004 and approximately 2 years later.[14] Knees were imaged in the sagittal plane on a 1.5-T whole-body magnetic resonance unit (Philips) using a commercial transmit–receive extremity coil as described.[14]

Assessment of BML

BML were defined as areas of increased signal intensity adjacent to subcortical bone present in either the medial or lateral distal femur or proximal tibia T2-weighted images.[6] Two trained observers, blinded to patient characteristics, together assessed the presence of lesions for each subject.[6] The presence of BML was determined. A lesion was defined as "large" if it appeared on two or more adjacent slices and encompassed at least one quarter of the width of the tibial or femoral cartilage being examined from coronal images, comparable to the previously described "grade 2" BML by Felson *et al*.[6] Lesions were further classified as "very large" if they appeared on three or more slices, comparable to the lesions described as "grade 3" by Felson *et al*.[6] The reproducibility for the determination of BML was assessed using 60 randomly selected knee MRI (κ value 0.88, $p<0.001$). If an individual had more than one BML underlying a cartilage plate or within the knee, the BML of the highest grade was used for analysis.

Assessment of cartilage defects

Cartilage defects were graded on the magnetic resonance images in the medial and lateral tibial and femoral cartilages using a validated classification system.[15–17] The cartilage defect score for a cartilage plate was defined by the most severe cartilage defect present, graded as follows: grade 0, normal cartilage; grade 1, focal blistering and intracartilaginous low-signal intensity area with an intact surface and bottom; grade 2, irregularities on the surface or bottom and loss of thickness of less than 50%; grade 3, deep ulceration with loss of thickness of more than 50%; grade 4, full-thickness cartilage wear with exposure of subchondral bone. A cartilage defect had to be present in two or more consecutive slices. The most severe cartilage defect present in the cartilage plate was used for analysis. The cartilage defect score for a compartment was calculated by summing the grades of the most severe cartilage defect in the tibial and femoral cartilage plates in that compartment. The medial and lateral scores were summed to obtain the total tibiofemoral cartilage defect score. Cartilage defect scores were read blind to sequence. Intraobserver reliability (expressed as intraclass correlation coefficient) was 0.90 for the medial tibiofemoral compartment and 0.89 for the lateral tibiofemoral compartment.[3] Change in cartilage defects was classified as to whether the defect score progressed (ie, increased score), regressed (ie, reduction in score) or remained stable (ie, no change in score).

Table 2 Number of subjects in whom cartilage defects progressed (deteriorated), remained stable, or improved (regressed) depending on whether a "large" or "very large" BML was present at baseline

	Large BML N = 22	No large BML N = 126	p Value*	Very large BML N = 8	No very large BML N = 140	p Value*
No of subjects in whom cartilage defects						
Progressed/deteriorated	10	55	0.84	5	60	0.35
Remained stable/no change	10	57		2	64	
Regressed/improved	2	15		1	16	

*Ordinal regression. BML, bone marrow lesion.

Table 3 Odds of progression of tibiofemoral cartilage defect score according to whether a "large" BML or a "very large" BML was present

	Univariate OR	p Value	Multivariate OR*	95% CI*	p Value
Medial tibiofemoral cartilage defect score					
Large BML	0.98	0.97	2.25	0.46 to 11.06	0.32
Very large BML	5.44	0.17	31.4	1.60 to 607	0.02
Lateral tibiofemoral cartilage defect score					
Large BML	0.67	0.57	1.35	0.30 to 6.20	0.70
Very large BML	1.24	0.82	2.09	0.30 to 14.75	0.46
Total tibiofemoral cartilage defect score					
Large BML	1.08	0.88	2.88	0.96 to 8.68	0.06
Very large BML	2.22	0.29	5.55	1.04 to 29.6	0.045

*Multivariate analysis adjusted for age, body mass index and initial bone area of the tibial plateau and initial cartilage defect score of the respective compartments. BML, bone marrow lesion; OR, odds ratio.

Cartilage volume measurement

The volumes of the medial and lateral tibial cartilage plates were measured using image processing on an independent workstation using the software program Osiris (University of Geneva). A trained observer read each MRI blinded to the timing of images. Independent measures of volume were made in a blinded fashion by a second trained observer.[18 19] The coefficients of variation for the medial and lateral cartilage volume measures were 3.4% and 2.0%, respectively.[18 19] Annual change in cartilage volume was calculated as (follow-up cartilage volume subtracted from initial cartilage volume) divided by the period of time between MRI scans.[18]

Bone area measurement

Medial and lateral cross-sectional areas of tibial plateau were determined by creating an isotropic volume from the input images that were reformatted in the axial plane. Areas were directly measured from these images. Coefficients of variation for the medial and lateral tibial plateau areas were 2.3% and 2.4%.[18]

Statistical methods

Baseline characteristics were compared between subjects in whom BML (at least large or very large) were present and absent, using unpaired t-tests for continuous variables and Somer's d-test for ordinal variables. Ordinal logistic regression was used to determine whether the presence or absence of a BML predicted cartilage defect progression, stability or regression. Multiple logistic regression was used to determine the odds of cartilage defect progression versus regression/stability, and of cartilage defect regression versus stability/progression depending on whether a BML was present in the compartment being examined. The distribution of annual change in cartilage volume was examined for normality. Once confirmed, linear regression was used to determine whether there were differences in change in cartilage volume in subjects with and without large or very large BML in the medial and lateral tibiofemoral compartments, respectively, and in the total tibiofemoral joint after adjusting for potential confounding. A p value less than 0.05 (two-tailed) was regarded as statistically significant. All analyses were performed using the SPSS statistical package (version 15.0.0).

RESULTS

Of 176 women recruited to this study, 148 women completed the follow-up (84.1%). Twenty-eight women did not undergo a second MRI because of death (n = 1), migration (n = 1), knee injury (n = 4), surgery (n = 1), withdrawal of consent (n = 3) and being unable to be contacted (n = 18). Apart from being younger (mean age 49.5 years (SD 6.3), p = 0.02 for difference) the characteristics of these women were similar to those who completed the study.

Table 1 provides the baseline characteristics of the 148 women who completed the study. BML were present in 22 women (14.9%), including eight "very large" BML. There were 11 BML in the medial compartment, of which three were "very large". In the lateral compartment, there were 11 BML, of which five were "very large". Subjects with "large" BML had a significantly larger lateral tibial plateau area (p = 0.03), total tibial plateau area (p = 0.04) and higher cartilage defect scores in all compartments ($p \leq 0.001$) than those without BML.

Are BML associated with the progression or regression of cartilage defects?

The number of knees in which the total cartilage defect score progressed, remained stable or regressed according to whether a "large" or "very large" BML was present at baseline is presented in table 2.

"Very large" BML were associated with an increased risk of progression of total tibiofemoral cartilage defects (odds ratio (OR) 5.55, 95% CI 1.04 to 29.6, p = 0.045) after accounting for age, BMI, initial defect score and bone area (table 3). Although large BML were associated with a tendency towards the total tibiofemoral cartilage defect score to progress (OR 2.89, 95% CI 0.96 to 8.68, p = 0.06), after accounting for the same factors, the strength of this finding weakened. The medial and lateral compartments were examined separately. When a "very large" BML was present in the medial compartment, cartilage defects in that compartment were more likely to progress (OR 31.4, 95% CI 1.60 to 607, p = 0.02; table 3). Similar results were obtained when large BML were excluded from the analysis (results not shown). These significant effects were independent of the initial cartilage defect score, which was the most significant determinant of the risk of progression of cartilage defects.

Whether BML were associated with the risk of cartilage defects improving (regressing) was examined. "Large" BML were associated with a tendency for cartilage defects to be less likely to improve (regress), but rather to remain stable or progress in the total tibiofemoral compartment (OR 0.27, 95% CI 0.05 to 1.60, p = 0.15). Although the direction of all point estimates suggested a protective effect in which there were

Table 4 Annual change in tibial cartilage volume depending on whether a "large" BML or a "very large" BML was present

	Univariate regression coefficient	p Value	Multivariate regression coefficient	95% CI*	p Value
Annual change in medial tibial cartilage volume					
Large BML	4.40	0.77	−0.6	−30.6 to 29.3	0.97
Very large BML	−0.30	0.99	7.9	−46.9 to 62.8	0.26
Annual change in lateral tibial cartilage volume					
Large BML	35.8	0.016	39.2	11.1 to 67.2	0.007
Very large BML	22.5	0.30	29.1	−12.8 to 70.9	0.17
Annual change in total tibial cartilage volume					
Large BML	10.4	0.56	11.0	−23.5 to 45.6	0.53
Very large BML	22.1	0.43	31.0	−23.2 to 85.1	0.26

*Adjusted for age, body mass index, initial cartilage volume and bone area for the respective tibial cartilage. BML, bone marrow lesion.

sufficient BML to examine this question, these did not reach statistical significance (data not shown).

Are BML associated with change in cartilage volume?

The relationship between BML and annual change in cartilage volume in the medial, lateral and total tibial cartilage was examined (table 4). In the lateral compartment, univariate analysis suggested "large" BML were associated with a higher loss of cartilage volume ($p = 0.02$; table 4), such that when a "large" BML was present at baseline, cartilage loss occurred at a rate of 35.8 mm^3/year higher than when no BML was present. After adjusting for age, BMI, initial cartilage volume and bone area, the significance of this difference increased (regression coefficient, r = 39.2 mm^3/year, 95% CI 11.1 to 67.2, $p = 0.007$; table 4). This trend persisted when only "very large" BML were examined, although the magnitude of difference and significance was diminished (r = 29.1 mm^3/year, 95% CI −12.8 to −70.9, $p = 0.17$). No significant effect of BML (either "large" or "very large") on annual change in medial and total tibial cartilage was found (table 4). Similar results were obtained when "large" BML were excluded from the analysis (results not shown).

DISCUSSION

This study examined the relationship between BML and changes in knee cartilage in healthy women. In these women, when BML were present at baseline, tibiofemoral cartilage defects tended to be more likely to develop or become more severe. This relationship was stronger when only larger BML were considered. Also, when a BML was present in the lateral compartment, more rapid loss of lateral tibial cartilage was seen.

This is the first longitudinal study examining whether, in pain-free knees, BML are associated with a change in cartilage. In individuals with osteoarthritis, BML have been demonstrated to predict increased progression of cartilage defects and loss of cartilage volume.[7 8] Increases in BML size have been associated with changes in urinary markers of cartilage metabolism.[20] Our results related to cartilage defects were also stronger when a larger BML was present. In a healthy population, without knee pain, change in cartilage defects may be more sensitive than change in cartilage volume, accounting for the weaker relationship seen between BML and cartilage volume. These data thus suggest that the relationship between BML and cartilage changes is similar in knees with established symptomatic osteoarthritis as well as in knees without clinical knee osteoarthritis or pain. This supports the hypothesis that the structural changes in knee osteoarthritis may be viewed as a continuum, extending across the spectrum from the healthy joint to one with established knee osteoarthritis. Conversely, these data provide evidence against the construct that BML in asymptomatic women represents a different process to that seen in knee osteoarthritis.[12]

Whether BML directly cause changes in articular cartilage or whether a common exposure such as microtrauma or obesity affects both BML and change in cartilage is unknown. Nevertheless, BML may act as a marker for an increased risk of progression of cartilage defects in a pain-free population. Identifying factors that affect the progression of cartilage defects in a healthy population is important because cartilage defects have been proposed as an early marker of knee osteoarthritis;[21] their presence predicts more rapid loss of articular cartilage in healthy individuals and predicts joint replacement in those with osteoarthritis.[15 17] Whereas there is a tendency for defects to progress in a healthy population, approximately one third resolve.[22 23] Identifying factors that prevent defect progression and/or promote defect resolution will be important in preventing knee osteoarthritis. However, to date, only lower BMI has been associated with the resolution of cartilage defects.[22] Age and higher BMI have been associated with defect progression.[22 23] These data suggest that BML, especially very large ones, are associated with an increased likelihood of cartilage defects progressing. It may be useful to identify factors that predict BML and affect their resolution, especially before the development of established knee osteoarthritis. The natural history of BML in a pain-free population is currently unknown; it is possible that by modifying this, the natural history of cartilage defects will also be affected, either directly or indirectly.

The association of BML with the progression of cartilage defects in a healthy pain-free population thus requires further study. In osteoarthritis, the presence and change in the size of BML is associated with parallel changes in serum markers of cartilage breakdown.[20] It is possible that BML may be a useful therapeutic target in the prevention of knee osteoarthritis; by affecting the prevalence of BML we may be able to reduce or delay the onset of osteoarthritis and diminish the progression of cartilage defects. This suggests that a systemic approach may be feasible, with data suggesting that the presence of BML is susceptible to systemic factors such as medications and dietary intake. For example, the use of antiresorptive therapy

(bisphosphonates and oestrogen therapy) in postmenopausal women has been shown to be protective of BML.[24] More recent data suggest that dietary manipulation may also be helpful, given the recent findings that BML are more likely to be present when dietary vitamin C and fruit intake is low, and monounsaturated fatty acid, polyunsaturated acid and n-6 polyunsaturated acid intake is high.[9 25]

This study has a number of limitations. As only women were examined, these findings may not be generalisable to men. However, osteoarthritis is more prevalent in women and significant gender effects have been described in the knee structure. This may have improved our ability to demonstrate relationships. Although we cannot exclude radiographic osteoarthritis, none of the women had knee pain. These data would thus be generalisable to the population for whom the primary prevention of osteoarthritis would apply. The power of this study to show an effect was limited by the low prevalence of BML (14.9%). Therefore, although we were able to demonstrate significant relationships, albeit with wide confidence intervals, between BML and progression in cartilage defects in the medial and total tibial cartilages and change in lateral cartilage volume, we may have been unable to identify weaker relationships. It is emerging that a change in cartilage defects may be seen earlier than changes in cartilage volume in a healthy population. Therefore, an increased study duration may have enabled us to detect a stronger relationship between a change in cartilage volume and the presence of BML. Similarly, the low number of BML limited our power to show effects in both compartments. For example, we showed a significant relationship between BML and the progression of defects in the medial compartment only and between BML and lateral tibial cartilage loss. Although it is possible that these differences may be due to unmeasured differences (eg, biomechanical factors) between the compartments, it is also possible that with more cases we would have been able to demonstrate similar results throughout the knee. Despite this, the findings tended to be consistent, whether large or very large BML were examined as the predictor of change, and showed the same direction of effect in all cartilages examined. In this study we were not able to measure knee alignment, which may have affected our findings. However, recent work suggests that the relationship between BML and progression persisted after accounting for alignment.[7] The main strength of this study is that it examines a healthy population of women longitudinally, allowing the relationship between BML and cartilage pathology to be examined in the early or prediseased state. We have also used a very conservative method for grading BML and cartilage defects, such that changes were required on at least two adjacent magnetic resonance images. This ensured that only large lesions were included, in contrast to some previous studies that required an abnormality to be present in one slice only.[26 27]

These data suggest that BML in pain-free knees, especially larger BML, are associated with the progression of cartilage defects and possibly cartilage volume loss in a healthy population. This suggests that BML have predictive validity, and may be useful as a target for the prevention of knee osteoarthritis. Given that recent data suggest that BML are affected by systemic factors, such as medications and diet, this raises the possibility that a systemic intervention, such as dietary manipulation, may be successful in reducing the prevalence of knee osteoarthritis. Even a small delay in the onset of clinical disease would have a substantial impact on the morbidity related to osteoarthritis, the most common form of arthritis worldwide.

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