SYSTEMIC FACTORS, STRUCTURAL BIOMARKERS AND VITAMIN D TREATMENT IN KNEE OSTEOARTHRITIS

by

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Submitted in fulfillment of the requirements for the Degree of Doctor Medical Research

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University of Tasmania
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STATEMENT OF ORIGINALITY

I declare that this thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, 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 acknowledgment is made in the text of the thesis.

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STATEMENT OF ETHICAL CONDUCT

The research associated with this thesis abides by the international and Australian codes on human and animal experimentation, the guidelines by the Australian Government's Office of the Gene Technology Regulator and the rulings of the Safety, Ethics and Institutional Biosafety Committees of the University.

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STATEMENT OF CO-AUTHORSHIP

This thesis includes papers for which Xingzhong Jin (XJ) was not the sole author. XJ was the lead in the research of each manuscript; however, he was assisted by the co-authors whose contributions are detailed below.

Chapter 4

Jin, X. et al. (2015). Circulating C reactive protein in osteoarthritis: a systematic review and meta-analysis. Annals of the Rheumatic Diseases, 74, 4:703-10.

Author Contributions

XJ and CD designed the review protocol. XJ carried out the literature search. XJ and JRB contributed to study selection, data extraction and quality assessment. LB and PO provided statistical supports for meta-analysis. XJ performed the analyses and drafted the manuscript. All the authors contributed to the preparation of the manuscript.

Chapter 5

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Author Contributions

GJ designed and obtained funding for the original TasOAC study. Analyses were designed by XJ, CD and GJ. Analyses were conducted by XJ and XW with advice from CD, LB and

LL. XJ, CD, BA and LB contributed to data interpretation. XJ and CD drafted the article. All authors critically revised it for important intellectual content, and approve the final version the article.

Chapter 6

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XJ and CD performed a systematic literature search. CD, GJ, TW, FC and AW designed the study. CD, XJ, GJ, FC, AW, ZZ, WH, BA and XW collected the results. XJ, CD, GJ, LB and TW were involved in data analysis. All authors were involved in interpreting the data. All authors were involved in preparing and critically revising the report and approved the final version for publication.

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Author Contributions

XJ and BA performed a systematic literature search. GJ and FC designed the study. CD conceived the study idea that cartilage defects predict osteoarthritis outcomes. CD measured cartilage defects and supervised other MRI measurement. ZZ, JW, BA and XW collected the results. XJ, BA, GJ, and LB were involved in data analysis. All authors were involved in interpreting the data. All authors were involved in preparing and critically revising the report and approved the final version for publication.

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Publications Arising from the Thesis

Chapter 4

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ABSTRACT

Osteoarthritis (OA) is a multifactorial disease of the joints and is a leading cause of pain and disability in older adults. The knee is the most common joint affected by OA. Up to the date of this thesis, there are no approved disease-modifying treatments available for knee OA. Therefore, there is an urgent need for identifying modifiable risk factors for this disease. This thesis aims to use a mixed approach to investigate multiple aspects of the disease, including the roles of systemic risk factors in knee OA, the effects of vitamin D supplementation on disease progression, as well as the predictive values of magnetic resonance imaging (MRI) biomarkers for knee replacement.

Chapter 4 systematically reviews 32 studies on the relationship between serum C-reactive protein levels measured by a high sensitivity method (hs-CRP) and OA, as well as the correlation between circulating CRP levels and OA phenotypes. Serum hs-CRP levels in OA were modestly but statistically significantly higher than controls. The levels were significantly associated with pain and decreased physical function, but not radiographic OA. This suggests low-grade systemic inflammation may play a greater role in symptoms rather than radiographic changes in OA.

Chapter 5 describes the longitudinal relationship between adiposity and change in knee pain. Data from a population-based sample of older adults show that body mass index (BMI) is the most consistent correlate of knee pain. Fat mass is associated with non-weight-bearing knee pain suggesting systemic mechanisms are involved.

Chapter 6 investigates the effects of vitamin D supplementation versus placebo on knee pain

and knee cartilage volume in symptomatic knee OA patients with low vitamin D levels in a randomized clinical trial. Compared with placebo, vitamin D supplementation did not result in significant differences in change in MRI-measured tibial cartilage volume or knee pain score over 2 years. There were no significant differences in changes of tibiofemoral cartilage defects or bone marrow lesions; however, fewer of those receiving vitamin D had increases in bone marrow lesions.These findings do not support the use of vitamin D supplementation for preventing tibial cartilage loss or improving knee pain in patients with knee OA.

Chapter 7 describes the longitudinal associations between serum levels of estrogen, progesterone and testosterone and MRI knee structural changes in both males and females with symptomatic knee OA. For women, progesterone was associated with cartilage volume and estradiol levels were inversely associated with grades of bone marrow lesions (BMLs), while estradiol, progesterone and testosterone were inversely associated with effusion-synovitis volume. No consistent associations were observed for men. This suggests endogenous sex hormones may be protective for joint structural changes in women but not men, which may contribute to observed sex differences in knee OA.

Chapter 8 describes the independent association of MRI markers and total knee replacement (TKR) over 10.7 years in older adults from a general population. MRI markers studied included cartilage defects, BMLs, effusion-synovitis and meniscal pathologies. Cartilage defects, BMLs and meniscal tears, but not effusion-synovitis or meniscal extrusion in the right knee were independent predictors of TKR in either knee over 10.7 years. The presence of multiple pathologies increased the risk of TKR, suggesting that MRI structural markers are good predictors of rapid knee OA progression in the general population.

In conclusion, this series of studies indicate that knee OA is a complex disease that is associated with systematic factors such as low-grade inflammation, adiposity and sex hormones. Vitamin D supplementation does not significantly prevent knee cartilage loss and knee pain

in patients with symptomatic knee OA. MRI structural markers are good predictors of end-stage knee OA in the general population. Future study should continue to validate the utility of using a panel of MRI biomarkers in predicting clinical endpoints. When developing disease-modifying OA drugs (DMOADS), systemic and metabolic inflammation should be a potential treatment target. Further investigation is needed to examine the effects of exogenous hormone replacement therapy on MRI structural changes in women.

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NOMENCLATURE

25OHD	25-hydroxyvitamin D
AAOS	American Academy of Orthopaedic Surgeons
ACR	American College of Rheumatology
BMI	Body mass index
CRP	C-reactive protein
CV	Coefficient of variation
DALY	Disability-adjusted life year
DEXA	Dual energy X-ray absorptiometry
DMOAD	Disease modifying osteoarthritis drug
ER	Estrogen receptor
EULAR	European League Against Rheumatism
GNP	Gross national product
ICC	Intra-class correlation coefficient
IL	Interleukin

K/L	Kellgren-Lawrence
MCID	Minimal clinically important difference
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
NO	Nitric oxide
NSAID	Nonsteroidal anti-inflammatory drug
OA	Osteoarthritis
OARSI	Osteoarthritis Research Society International
PGE	Prostaglandin E
RCT	Randomized clinical trial
ROA	Radiographic osteoarthritis
TIMP	Tissue inhibitors of metalloproteinase
TKR	Total knee replacement
VDR	Vitamin D receptor
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index

CHAPTER 1

Introduction

1.1 Overview of osteoarthritis

Osteoarthritis (OA) is the most common form of arthritis. It is a chronic joint disease and commonly affects weight-bearing joints such as the knees, hips and spine, with the hands and neck also being frequently affected. Primary OA is an idiopathic disease associated with aging. The disease may be confined to one or two joints, or present in three or more joints in what is known as generalized OA. Secondary OA is caused by an underlying condition, such as injury, congenital joint abnormalities, surgery, diabetes and other hormone disorders. The course of secondary OA involves many similar disease processes as primary OA.

The definition of OA has evolved over the course of the past few decades and it is increasingly recognized that OA is a disease of the whole joint. Although its signature structural pathology is articular cartilage loss, many other surrounding joint structures are pathologically involved including subchondral bone, ligaments, menisci, periarticular muscles, peripheral nerves and synovium. The collective changes of these joint tissues result in symptoms of pain, stiffness and functional disability [1].

1.1.1 Epidemiology and economic burden

The prevalence of OA increases with age, in particular after the age of 45 years. The global age-standardized prevalence of knee OA was 3.8% and hip OA was 0.85%. Knee and hip OA is ranked as the 11th highest contributor of the 291 conditions to global disability and 38th highest in disability-adjusted life year (DALYs) [2]. In Australia, the burden of OA was ranked 23rd and the disease was estimated to affect over 1.8 million Australians with a prevalence rate of 8.0%, according to the 2011-2012 National Health Survey [3]. In industrialized countries, medical costs of OA account for 1.0% to 2.5% of the gross national product (GNP) [4]. In Australia, OA and other musculoskeletal conditions were the fourth most expensive disease group in the year 2008-2009, accounting for 9% ($5.7 billion) of the total health care expenditure. OA accounted for 29% of this expenditure ($1.6 billion) and the largest proportion (77%) were spent on hospital costs, mainly associated with knee and hip joint replacement [5]. OA is expected to become the fourth leading cause of disability by the year 2020 with increasing life expectancy and an aging population [6].

There are also indirect and intangible costs attributable to OA. The pain and disability which OA patients experience can lead to a loss of health and well-being, loss of leisure time and a decreased quality of life. This further contributes to indirect costs of OA through reduced work performance and productivity, increased absenteeism and loss of production to the economy as a result of the related disease morbidity [7]. A progressively aging population is likely to further add to the disease burden and the cost of related health service in Australia.

1.1.2 Symptoms and signs

Pain is the predominant symptom of OA that causes patients to seek medical advice [8]. The onset is gradual or insidious and the pain experienced is usually intermittent, typically wors-

ens during and after using the involved joints. Inflammatory flares can happen during the course of the disease. Initially, pain may be self-limited, pain at rest or during the night suggests advanced or severe OA. Patients also experience joint stiffness, tenderness, a sensation of instability with clinical signs such as crepitus, restricted range of joint movement, joint deformity and muscle weakness. These symptoms lead to both physical and psychological disability, and impaired quality of life [9]. Patients could experience major disability with daily living activities including walking, stair-climbing and housekeeping.

1.1.3 Risk factors

For decades, OA was thought to be a degenerative joint disease as the result of 'wear and tear' that happens in joints as people get older. Research over the last decades has shown evidence that OA is a multifactorial and complex disease affected by many systemic and local factors.

Age remains one of the strongest systemic risk factors for the development of OA. The prevalence of OA defined radiographically rises from nearly zero in young people to a striking rate of 29% in people aged 65 and over (Figure 1.1). There is a non-linear relationship between aging and OA. The incidence rate of symptomatic OA increases rapidly around age 50 and then levels off after age 70 (Figure 1.2). The increase in incidence and prevalence of OA with age is likely a consequence of several biologic changes that occur with aging, including a decreased repairing ability of chondrocytes, an increase in laxity of joint ligaments making older joint relatively unstable, and a failure of major shock absorbers or protectors of the joint. [10].

Women are more likely to have OA than men. Estimates from Australia indicate that the prevalence of OA is higher among women than men among all age groups [3]. Women are more often affected with OA of the hand, foot and knee than men [10] and women also have

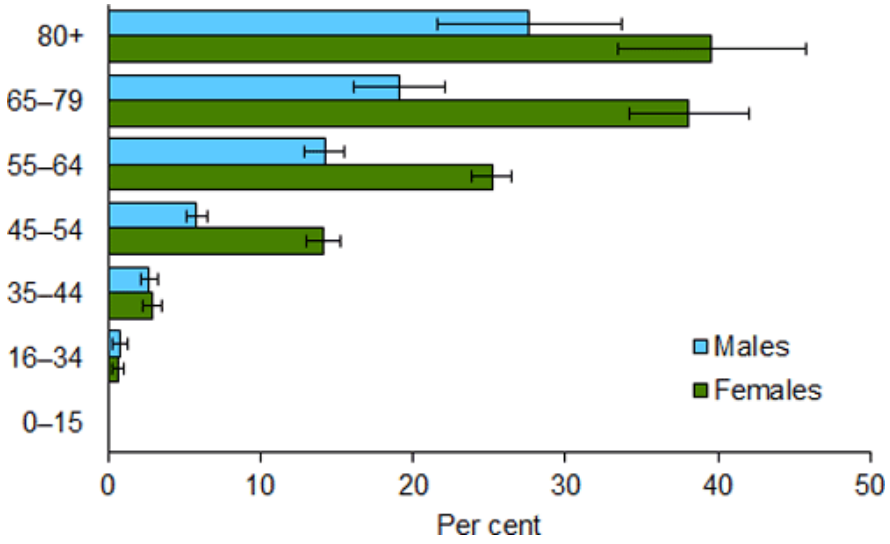


Figure 1.1: Prevalence of osteoarthritis in Australia, 2011–12.

Source: AIHW analysis of unpublished ABS Australian Health Survey, 2011–12

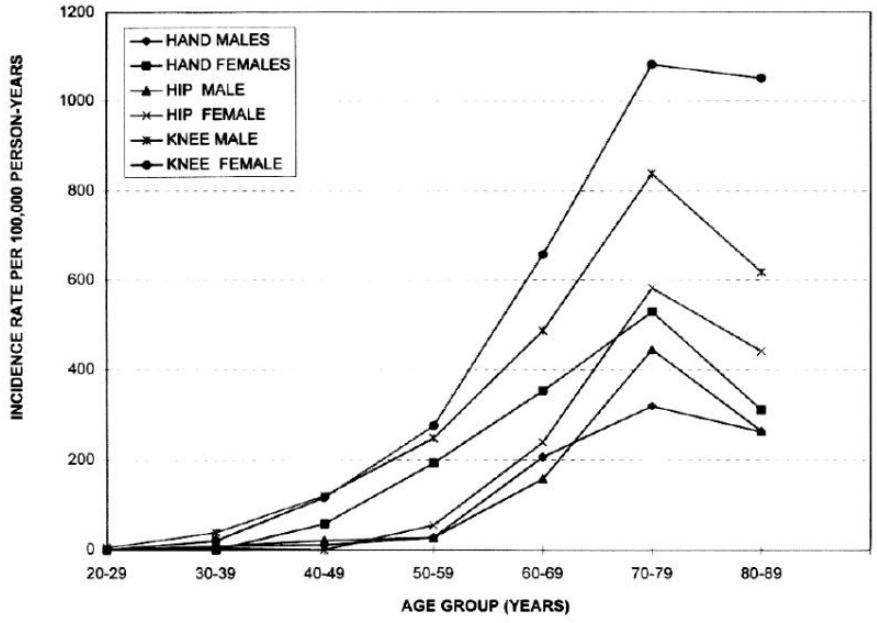


Figure 1.2: Incidence of osteoarthritis between males and females.

Source: D. T. Felson and Y. Zhang. "An update on the epidemiology of knee and hip osteoarthritis with a view to prevention". In: Arthritis Rheum. *41.8 (1998), pp. 1343–1355*

more severe OA [11]. Moreover, this sex difference in prevalence increases with age and the pattern suggests a role of post-menopausal hormone deficiency in increasing the risk of OA. The role of hormonal factors is reviewed in detail in Section 1.2.4.

Obesity or overweight is perhaps the strongest modifiable risk factor for the development of OA, especially for knee OA [12]. A 5-unit increase in body mass index is associated with an 35% increased risk of knee OA [13]. Obesity has also been shown to be a strong predictor of hand OA with an overall risk ratio of 1.9 [14]. The link between obesity and hand OA is particularly intriguing as it is suggestive of a systemic non-mechanical effect of body fat on OA through altered metabolic and hormonal profiles [15, 16]. Other systemic factors include chronic inflammation, vitamin D deficiency, ethnicity, congenital conditions and genetics.

Local risk factors for OA affects joints independently. While most local risk factors do not increase the odds of OA in all sites of the body, some may be shared by some joints. In addition to metabolic effects, obesity and overweight could lead to increased risk of OA through increases to mechanical loadings [17], particularly in the knee [18]. It is well understood that injury is a strong local risk factor for OA [19]. Injury can cause damages to a number of structural tissues, including cartilage, ligaments and meniscus, which can result in increased risk of development of hip and knee OA [20, 21]. Inflammation is usually induced during tissue repair due to injury, and the increase in inflammatory mediators may negatively affect cartilage metabolism, predisposing the subjects to OA [22]. Malalignment of the knee can increase the risk of OA progression by 4 to 5 times in the tibiofemoral compartment [23] or 2 times in the patellofemoral compartment [24]. Exercise is considered to have dual roles in OA. While mild to moderate non-painful exercise in the absence of existing joint condition is considered to have a beneficial effect [25], which may be mediated by muscle strengthening [26], high-intensive and long-term weight bearing exercise may be associated with increased risk of OA development [27].

1.1.4 Knee osteoarthritis

The knee is the most common joint affected by OA. Nearly one in two adults will develop symptomatic knee OA by the age of 85 with an estimated lifetime risk of 44.7% [28]. Knee OA is the most common cause of knee pain in people over 50 years old. Frequent knee pain affects approximately 25% of older adults [29] and it is the most important factor determining disability and a major reason for knee replacements in those with clinical knee OA [30]. The research conducted in this thesis focuses on knee OA.

Knee OA can be defined radiologically by structural pathology and diagnosed clinically using joints symptoms. Many studies combine both of these for the purpose of an epidemiologic investigation, using the term radiographic osteoarthritis (ROA) and symptomatic osteoarthritis.

Radiographic criteria

Conventional radiographs remain the gold standard for the diagnosis of knee OA. The first criteria for definition of ROA were introduced in 1957 by Kellgren and Lawrence [31]. The Kellgren and Lawrence (K/L) grading system is an ordinal 5-point scoring system that includes radiographic assessment of joint space narrowing (JSN) and osteophytes. The K/L scoring system is displayed in Table 1.1. In 1961, the K/L scoring system was accepted as standard criteria by the World Health Organization. Since then, it has been extensively used in epidemiological studies. Figure 1.3 highlights joint space narrowing and osteophytes on a radiograph.

Table 1.1: Kellgren and Lawrence (K/L) grading system

Definition grades	**Description**
Grade 0: Normal	No osteoarthritis
Grade 1: Doubtful	Doubtful narrowing of joint space and possible osteophytic lipping.
Grade 2: Mild	Definite osteophytes and possible narrowing of joint space.
Grade 3: Moderate	Multiple osteophytes, definite narrowing of joint space and some sclerosis and possible deformity of bone ends.
Grade 4: Severe	Large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone ends.

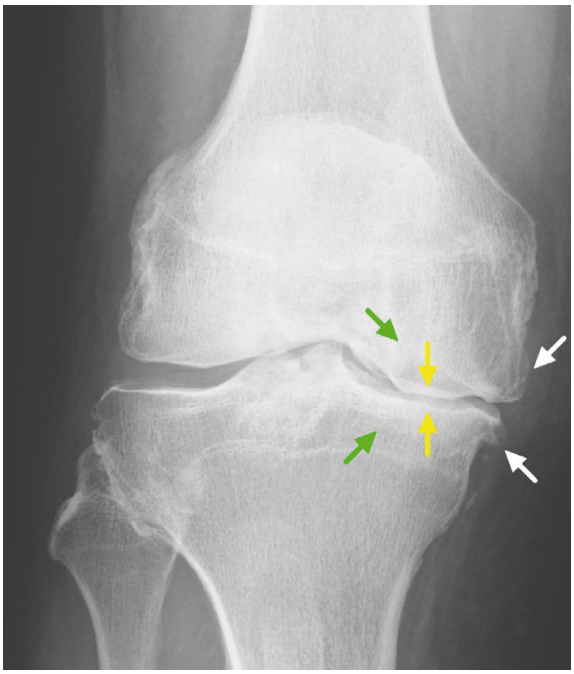


Figure 1.3: Joint space narrowing and osteophytes.

Joint space narrowing (yellow arrows), osteophytes (white arrows) and subchondral sclerosis (green arrows)
Source: A. Horng. "Gonarthrosis radiology: cross-sectional imaging in conventional diagnostic radiography". In: ASU International. *9.2 (2015)*

Clinical criteria

Although the presence of radiographic knee OA carries a definite predisposition to symptoms in the knee, there are potential limitations to the use of only radiographic criteria in clinical research studies, because some people with radiographic knee OA have no symptoms in the knee [33]. The American College of Rheumatology (ACR) defines symptomatic knee OA as frequent knee symptoms defined as ”pain, aching, or stiffness in or around the knee on most days” for at least one month during the past 12 months[34]. This definition has been used in many epidemiological studies.

1.1.5 Treatment and management

Despite the high prevalence and severe socioeconomic burden of knee OA, there are no registered disease-modifying osteoarthritis drugs (DMOADs) available to modify structural progression. Proven preventive strategies are also quite limited as its etiology is not well established. Current therapeutic treatments for knee OA are mostly palliative and focus on the relief of pain. According to the ACR guideline 2012 [35], analgesics such as acetaminophen [36, 37], oral or topical non-steroidal anti-inflammatory drugs (NSAIDs) [36] or more potent painkiller tramadol are conditionally recommended for the pharmacologic management of knee OA. Intraarticular injections with corticosteroid are recommended as an alternative approach if acetaminophen or NSAIDs does not have a satisfactory clinical response [38]. These treatments often are only moderately effective, with over 75% of patients reporting need for additional symptomatic treatment [39].

There is an emphasis on non-pharmacological management for knee OA including weight loss, education and exercise. Recent guidelines published by the American Academy of Orthopedic Surgeons (AAOS), ACR, the European league against rheumatism (EULAR)

and Osteoarthritis Research Society International (OARSI) consistently recommend weight management program for overweight patients with knee OA [40, 41, 35, 42]. A systematic review and meta-analysis found moderate weight reduction regime reduces pain and physical disability for overweight patients with knee OA [43]. An exercise program is also strongly recommended for all patients with symptomatic knee OA. A number of systematic reviews and meta-analyses have demonstrated small to moderate short-term benefits of aerobic, resistance land-based [44, 45, 46, 47] and water-based exercises [48, 49, 50].

As many as 40% of patients with knee OA have used at least one dietary supplement to treat their condition [51]. Dietary supplements containing cartilage synthesis precursors, such as glucosamine and chondroitin are among the most popular products for OA. However, the efficacy of these supplements are uncertain. Results from various systematic reviews showed small to non-existent effect in symptom relief and disease modification with a high degree of heterogeneity between included trials [52, 53, 54, 55, 56]. Therefore, they are not generally recommended in clinical practice. Although dietary vitamin D supplementation is not mentioned in the clinical guidelines, there is possible benefit to patients with knee OA and it is discussed in the Section 1.2.3.

Surgical interventions are generally reserved for patients with advanced stage of OA when non-surgical treatments are no longer effective [36]. Arthroscopic lavage and debridement smooths out the rough portion of cartilage or meniscus and removes loose fragments of tissue. However, the evidence from systematic reviews shows that arthroscopic surgery is not effective for managing knee OA, because the inconsequential benefit of arthroscopic surgery is limited in time and absent at one to two years after surgery [57, 58, 59]. Osteotomy involves the removal of bone to reshape joints to correct malalignment [40]. Knee replacement is the removal of large portions of bone and cartilage and substitution with artificial articulating surfaces. Knee replacement improves both symptoms and function and is indicated for end-stage knee OA [60].

1.2 Systemic factors of knee OA

Knee OA is a disease process driven by multiple risk factors. Some well-known risk factors for knee OA including aging, obesity, gender, and genetic factors have been extensively studied. This section reviews the literature of less well-established risk factors for knee OA that could impact the knees through their effects on systemic metabolism rather their contribution to the local joint structures. These systemic risk factors include obesity, low-grade systemic inflammation, vitamin D deficiency and sex hormones.

1.2.1 Obesity

Knee OA and obesity are the two conditions whose prevalence are accelerating worldwide [61]. The increasing prevalence of knee OA is likely linked to the increasing proliferation of obesity. It is now recognized that knee OA exists in the highly metabolic environment of obesity.

Obesity is usually defined as having a body mass index (BMI) more than 30 kg/m^2. Although the definition of obesity based on BMI was not widely adopted until the 1990s [62], obesity has long been recognized as a risk factor for prevalent OA, especially knee OA [63, 64, 65]. Data from the Chingford study suggested that women in the highest tertile of BMI had six-fold increased odds of knee OA and nearly 18 times higher odds of bilateral knee OA, compared with women in the lowest tertile of BMI [66]. More recently, a systematic review of 23 cohort studies demonstrated that all studies were consistent in reporting obesity as a risk factor for the onset of knee OA in older adults with a pooled odds ratio (OR) of 2.66 [67].

In addition to the mechanical impacts on the knees, it is increasingly recognized that the inflammatory environment associated with obesity contributes to the development and pro-

gression of knee OA. Adipose tissue, once considered a passive storage portal of energy, is now recognized as a highly metabolic endocrine organ with the capacity to secrete active agents including adipocytokines, such as leptin, resistin, adiponectin and visfatin [68]. In patients with knee OA, leptin, adiponectin and resistin levels have been detected in the synovial fluid [69, 70]. Adipocytokines acting in an autocrine or paracrine manner regulate the expression of proteolytic enzymes such as metalloproteinases (MMPs) as well as inflammatory cytokines, nitric oxide (NO) and prostaglandins (PGEs). Leptin is positively correlated with BMI, body weight and fat mass percentage. Leptin was shown to be pro-inflammatory as it enhanced NO synthesis, PGE2, interleukin-6 (IL-6) and IL-8 secretion in knee OA cartilage [71]. The presence of the soluble leptin receptor is associated with reduced cartilage synthesis and increased cartilage degradation marker in knee OA [72]. Adiponectin may have a protective effect on knee OA. In vitro, Chen and colleagues showed the protective effect of adiponectin on articular cartilage by up-regulating the tissue metalloproteinase inhibitor (TIMP-2) and decreasing IL-1β mediated MMP-13 expression [70]. In patients with knee OA, adiponectin levels in both plasma and synovial fluid decreased significantly as the severity of OA increased [73] and the adiponectin to leptin ratio in synovial fluid was found to predict reduced knee pain [74].

However, not all obese persons develop knee OA, nor are all individuals with knee OA obese [75]. Because BMI is calculated based on weight and height, it is only a surrogate measure of obesity which cannot discriminate fat and lean mass. It is increasingly clear that BMI is a rather poor indicator of the percentage of body fat mass. Indeed, waist-to-hip and waist circumference were better measures of central adiposity and were better predictors of knee OA incidence than BMI [76]. Also, fat mass and muscle lean mass had a better statistical fit than BMI to explain the odds of having and the severity of knee OA [77]. Furthermore, Ding and colleagues suggested that body fat was a better predictor than BMI for tibial cartilage loss. Body fat mass and muscle lean mass may have opposite effects on knee cartilage loss

[78] and knee pain [79]. While body fat mass was associated with more cartilage volume loss and increased knee pain, muscle lean mass appeared to be protective. Similar detrimental effect of fat mass and a beneficial effect of lean mass on knee cartilage volume was found in healthy adults [80]. Cicuttini and colleagues also showed that reduced lean mass was associated with reduced tibial cartilage volume [81]. Taken together, these studies suggest that body composition, in addition to excessive body weight measured by BMI, may play an important role in the structural and symptomatic changes of knee OA. However, neither of two longitudinal studies showed a significant relationship between body composition and tibiofemoral cartilage defects or the progression of tibiofemoral cartilage defects [80, 81]. In a systematic review and best evidence analysis, Mezhove and colleagues suggested that larger and longer cohort studies are required to understand the role of body fat distribution in the risk of knee OA, because of a limited number of cohort studies having significant findings between obesity and knee cartilage [82].

1.2.2 Inflammation

Knee OA has traditionally been classified as noninflammatory arthritis. This belief was based on the observation that there are fewer leukocytes in knee OA synovial fluid compared to rheumatoid arthritis, reactive arthritis and septic arthritis. Moreover, the only cell type present in cartilage, chondrocytes, have very low metabolism activity with no ability to repair cartilage. Once the articular cartilage is damaged, it cannot respond to the damage by a usual inflammatory response because it is non-vascularized and non-innervated [83]. This paradigm has been shifted in the last few decades as inflammation is increasingly recognized as an important contributor to the symptoms and progression of knee OA [84]. The discovery that inflammatory mediators such as cytokines or prostaglandins can increase the production of MMPs by chondrocytes brought about the inflammatory theory. Many studies have observed the presence of synovitis under arthroscopy, magnetic resonance imaging (MRI) or

ultrasonography in knee OA and it is associated with increased risk of radiographic knee OA [85, 86]. The mechanism leading to synovitis in knee OA remain controversial [87]. The most accepted hypothesis is that joint trauma or overuse triggers cartilage degradation, then cartilage fragments fall into the joint and contact the synovium. Because the cartilage debris is considered as foreign bodies, synovial cells react by producing inflammatory mediators and releasing them in synovial fluid. These mediators can activate chondrocytes present in the superficial layer of cartilage, which leads to MMP synthesis and eventually increase cartilage degradation. Theses inflammatory mediators also induce synovial angiogenesis and increase the production of inflammatory cytokines and MMPs by synovial cells. Tissue damage, synovial activation, inflammation and cartilage degradation turn into a self-perpetuating vicous cycle.

Additionally, inflammation occurring within the joint tissue may be reflected outside the joint in patients with knee OA. C-reactive protein (CRP) is an acute-phase protein that is produced by hepatocytes and regulated by the pro-inflammatory cytokine interleukin (IL)-6 in response to both acute and chronic inflammation. It has a constant half-life of 19 hours so the sole determinant for circulating CRP level is the synthesis rate [88]. This unique property of CRP makes it an excellent marker used to measure chronic systemic inflammation. One of the key epidemiology studies conducted by Specter and colleagues demonstrated that serum levels of CRP are strongly associated with the development and progression of knee OA [89]. Another study by Pearle and colleagues showed a positive correlation between serum CRP levels and histological evidence of synovitis and synovial fluid IL-6 at the time of joint replacement [90]. Other studies showed that the levels of several inflammatory mediators were higher in OA than healthy serum [91, 92]. These observations strongly suggest that the systemic inflammation observed in knee OA is at least partially reflective of local synovial inflammation.

The source and type of inflammation may differ by OA phenotypes. Bijlsma and colleagues

proposed the division of OA into distinguishable phenotypes [93]. In post-trauma phenotype, damage of the cartilage causes the triggered chondrocytes to release pro-inflammatory autocrine factors, which in turn trigger a local inflammatory response (synovitis) that accelerates the cartilage breakdown. This inflammatory activity is enhanced by the accelerated release of catabolic cartilage constituent [94, 95]. In the aging phenotype, inflammation is triggered by external mediators such as cytokines and proteases, as well as internal cellular mechanisms leading to increased production of inflammatory mediators and lack of elimination of oxidated proteins. These proteins will, in turn, increase the concentration of reactive oxygen species (ROS) in cells, further adding to the oxidative damage triggering the inflammation [96]. Interestingly, oxidative stress can promote cell senescence, and in particular chondrocyte senescence [97]. In obesity phenotype, the inflammatory theory could be explained by adipokines that are released mainly by abdominal adipose tissue [98]. Systemic adipokines were found associated with local synovial tissue inflammation [99] and serum adipokine concentration was associated with OA severity [100, 101]. Interestingly, recent clinical studies have suggested that metabolic syndrome (MetS) rather than obesity itself has the greatest impact on the initiation and severity of knee OA [102, 103, 104]. Lastly, calcium pyrophosphate dihydrate and basic calcium phosphate crystals are common in knee OA joint fluids and tissues [105]. These crystals may trigger innate immune responses leading to catabolic responses in chondrocytes and synovitis [106]. Figure 1.4 shows a diagram for the role of inflammation in the pathogenesis of knee OA according to phenotype.

1.2.3 Vitamin D

Vitamin D is a fat-soluble secosteroid comprising two major molecules, vitamin D_2 and vitamin D_3. Sun exposure is the major way to produce vitamin D in most humans [107]. Very few foods in nature contain vitamin D, such as the flesh of fatty fish and fish liver oils [108]. Vitamin D from the skin and diet is circulated to the liver and converted to

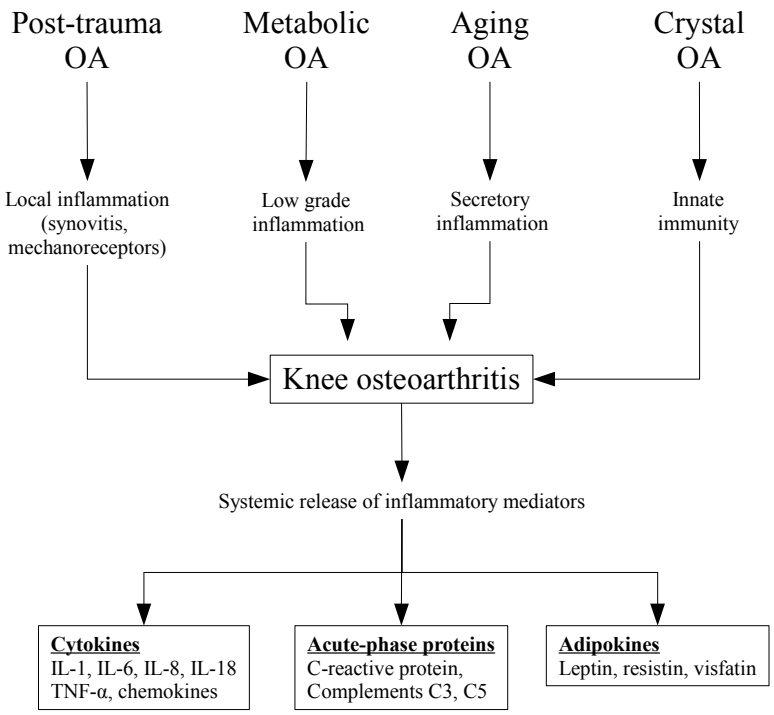


Figure 1.4: The role of inflammation in the pathogenesis of knee OA according to phenotype.

For each phenotype, the main pathway leading to the release of inflammatory mediators by the joint is highlighted. However, some pathways are shared between phenotypes.

the prohormone calcidiol, or 25-hydroxyl vitamin D (25OHD), which is the most reliable indicator of vitamin D status [109].

It is well known that vitamin D has important roles in bone health [110]. Vitamin D deficiency not only causes rickets among children [111] but also precipitates and exacerbates osteoporosis among adults and causes the painful bone disease osteomalacia [112]. In recent decades, epidemiological studies suggested serum vitamin D levels were associated with the risk of cancers [113], autoimmune diseases [114], type 2 diabetes [115], and cardiovascular diseases [116].

Vitamin D deficiency, defined as a serum concentration less than 50 nmol/L, is very common

in older people. It is estimated that 40 to 100% of U.S. and European elderly men and women are deficient in vitamin D [107]. High rates of vitamin D deficiency have also been reported in Australia, especially in the southern states including South Australia, Victoria and Tasmania [117, 118, 119]. Knee OA frequently co-exists with vitamin D deficiency in older adults, suggesting a link between them [120].

In vitro study has demonstrated that vitamin D receptors (VDRs) are expressed in human articular chondrocytes in osteoarthritic cartilage and $1\alpha,25-(OH)_2D_3$ may regulate the expression of MMPs and PGE2 in chondrocytes via VDRs [121]. It is hypothesized that vitamin D could reduce bone turnover and cartilage degradation, thus preventing the development and progression of knee OA [122, 123].

Epidemiological studies have shown evidence that supports a link between vitamin D and knee OA. In the Framinghan study, dietary intake and serum levels of vitamin D were found associated with progression of knee OA [124]. In the Rotterdam study, low dietary vitamin D intake increased the risk of progression of knee ROA [125]. Recent data from the Osteoarthritis Initiative (OAI) study suggested that individuals deficient in vitamin D had an increased risk of knee OA progression [126]. Also, vitamin D deficiency was cross-sectionally associated with knee pain [127] and was an independent predictor of worsening knee pain in older adults [128]. All these suggest that correction of vitamin D deficiency using supplementation could be beneficial in knee OA. However, results from two randomized controlled trials (RCTs) appear controversial [129, 130]. Both RCTs have limitations. The first RCT included patients without vitamin D deficiency who may not benefit from vitamin D supplementation and patients whose disease was too severe to respond to vitamin D treatment. Also, it had a small sample size [129]. The latter RCT did not examine structural changes and had one-year follow-up, which may be too short to observe disease progression [130]. Further clinical trials are needed to address these limitations in methodology and confirm the benefit of vitamin D supplementation for knee OA.

Table 1.2: Cohort studies for the association between 25OHD and knee OA

Study	Assessment of OA	Follow-up (years)	Adjustment	Results
Ding 2009 [120]	MRI tibial cartilage volume loss	2.9	Age, sex, BMI, smoking, steps per day, knee pain, cartilage defects, season, tibial bone area, ROA and disease status	Baseline 25OHD was associated with reduced cartilage volume loss; vitamin D deficiency predicted increased cartilage loss.
McAlindon 1996 [124]	Modified KL score, JSN and OP	8	Age, sex, BMI, weight change, physical activity, knee injury, energy intake and health status	Lower tertile of 25OHD predicted knee JSN, OP and ROA progression but not incident ROA.
Bergink 2009 [125]	KL score, JSN and OP	6.5	Age, sex, BMI, BMD, smoking, health status, disability index, fall tendency, baseline JSN and season	Lowest tertile of 25OHD was associated with incidence of JSN in women and progression of ROA.
Konstari 2012 [131]	Definite and probable knee OA diagnosed by physician	22	Age, sex, BMI, education, physical workload, smoking, leisure physical activity, injuries and season	No association between serum 25OHD and the incidence of knee OA, but the effects differ in season.
Felson 2007 [132]	KL score, JSN and OP; MRI WORMS assessment for cartilage loss	9.5 or 2.5	Age, sex, BMI, weight change and baseline KL score	25OHD was not associated with radiographic worsening (JSN and OP) and cartilage loss.
Zhang 2014 [126]	Incidence and progressive knee JSN	2	Age, sex, ethnicity, BMI, physical activity, alcohol intake, smoking, dietary vitamin D and calcium, season, and knee injury/surgery	Low 25OHD ($<$15ng/ml) increased the risk of JSN progression by $>$2-fold compared to greater 25OHD concentrations.

ROA, radiographic osteoarthritis; JSN, joint space narrowing; OP, osteophyte; BMD, bone marrow density; WORMS, Whole-Organ Magnetic Resonance Imaging Score.

Table 1.3: Comparison of two randomized controlled trials on vitamin D for knee OA

	McAlindon 2013 [129]	Sanghi 2013 [130]
Participants	146 patients with symptomatic knee OA	107 patients with knee OA with vitamin D insufficiency
25OHD level	55.7±24.7 nmol/L	<50 nmol/L
Intervention	Oral vitamin D3 2,000IU/day	Vitamin D2 60,000IU/day for 10 days followed by 60,000IU/month
Follow-up	2 years	1 year
Outcomes	WOMAC for pain; cartilage volume loss assessed using MRI	WOMAC, VAS for pain; KL scoring for radiographic OA
Conclusion	Vitamin D had no symptom or structure-modifying benefits for knee OA	Vitamin D intake was beneficial in improvement of knee pain and physical function in knee OA; no significant difference in radiological features in both groups

1.2.4 Sex hormones

Sex hormones have long been considered a possible systemic factor for OA, especially in women [133, 134]. The prevalence of knee OA in men and women are similar up to the age of 50, however, after menopause women have a significantly higher prevalence of knee OA than age-matched men [135, 136], suggesting an influence of hormonal factors in the pathogenesis of the disease. The relationship between hormonal changes due to menopause and OA was first described in a group of women with Herberden's nodes characterized by a rapid onset of symptoms and multiple joint involvement. The term 'menopausal arthritis' was used to described the condition and was later changed to 'primary generalized osteoarthritis' [137].

Numerous studies have been carried out to evaluate the relationship between estrogen and the development of knee OA. Two types of estrogen receptor (ER) are widely but differentially

expressed in most tissues and organs [138]. $ER-\alpha$ is highly expressed in the hypothalamus, where it plays a pivotal role in regulating food intake and energy expenditure by estrogens. $ER-\beta$, on the other hand, has an important role in tissue homeostasis such as bone and cartilage stabilization [139]. Estrogen has been demonstrated by in vitro studies to have protective effects on articular cartilage [140, 141, 142]. Moreover, the protective effect of estrogen appears to be sex-dependent as the chondroprotective effect was found only in female chondrocytes [141, 143, 144].

However, the findings from epidemiological studies regarding the risk of knee OA in relation to endogenous and exogenous sex hormones are conflicting. In the Southeast Michigan Arthritis Cohort, women having lower baseline concentrations of endogenous estradiol had an increased risk of developing radiographic knee OA [145]. Some studies have shown that women receiving estrogen therapy had a lower risk of developing radiographic knee OA and that the protective effect increased with increasing duration of estrogen therapy [135, 146, 147]. The results from the Framinghan Study showed that current use of estrogen replacement therapy had a moderate, although not statistically significant, protective effect against worsening of radiographic knee OA [148]. Similarly, the findings from the Women's Health Initiative also showed that women taking estrogen with more than 80% compliance had a 50% reduction in total knee joint replacement [149]. In contrast, there are other studies which have shown no effects of post-menopausal hormone replacement on knee OA. In a randomized clinical trial in a group of older post-menopausal women with heart disease, no significant difference was found in terms of knee pain or associated disability between those who were on estrogen plus progestin therapy and those taking placebo [150]. In a recent systematic review, no clear association was observed between female sex hormones and knee OA [151], suggesting that relationship may be too complex or that other unknown mechanisms may play a role in the increased prevalence of knee OA in menopausal women.

In summary, while there is a growing body of evidence that suggests a protective effect

of estrogen on cartilage and radiographic knee OA, it remains uncertain whether it has a beneficial effect on MRI structural biomarkers or total knee replacement due to a lack of long-term studies. Hormone replacement therapy is currently not recommended as a first-line treatment against the progression of knee OA, considering that the health risks of HRT may outweigh the potential benefit [152].

1.3 MRI structural biomarkers

The current imaging standard for evaluating structural changes associated with OA is plain radiography. Although plain radiography, which mainly focuses on joint space narrowing and osteophytes, is useful for the diagnosis of knee OA, it is only weakly associated with clinical symptoms and it is a poor predictor of cartilage loss and total knee replacement [153]. Plain radiography is also not sensitive to structural changes. By the time when structural changes are present on a radiograph, an estimate of over 10% of knee cartilage has been lost and over 40% patients have had cartilage defects [154]. In addition, joint space narrowing observed on knee radiograph does not distinguish cartilage volume loss and meniscal pathologies [155].

MRI has become an increasingly popular research tool for evaluating structural changes of knee OA in recent decades. The main advantage of MRI over traditional knee radiography is the ability to perform a holistic, three-dimensional assessment of early structural changes that are not discernible on x-ray. MRI can show the direct visualization of cartilage, subchondral bone, effusion-synovitis, menisci and other joint tissues in multiple tomographic planes. MRI can detect the presence of OA with high specificity and moderate sensitivity compared with radiography and arthroscopy [156]. The most promising MRI structural biomarkers identified in systematic reviews with respect to reliability, responsiveness and validity, were quantitative cartilage morphometry, cartilage defects and bone marrow lesions

on semi-quantitative analysis [157]. These structural pathologies were found associated with clinical and structural progression of knee OA [158, 159, 160, 161, 162]

1.3.1 Cartilage volume

Loss of cartilage is the primary feature of knee OA progression. Comparative analysis studies demonstrated that MRI-based knee cartilage volume measurement is highly accurate with an error of less than 10% compared to the volume estimated by means of water displacement [163, 164, 165]. Regardless of the health status of the patients, the coefficient of variation (CV) is less than 5% for intra-observer reliability and up to 7.8% for inter-observer reliability [165, 166]. These studies demonstrated that knee cartilage volume can be accurately and reproducibly measured by the same scanner and a single experienced reader. Various methods have been developed to semi-automatically measure the whole cartilage volume (Figure 1.5) in the knee [167, 168].

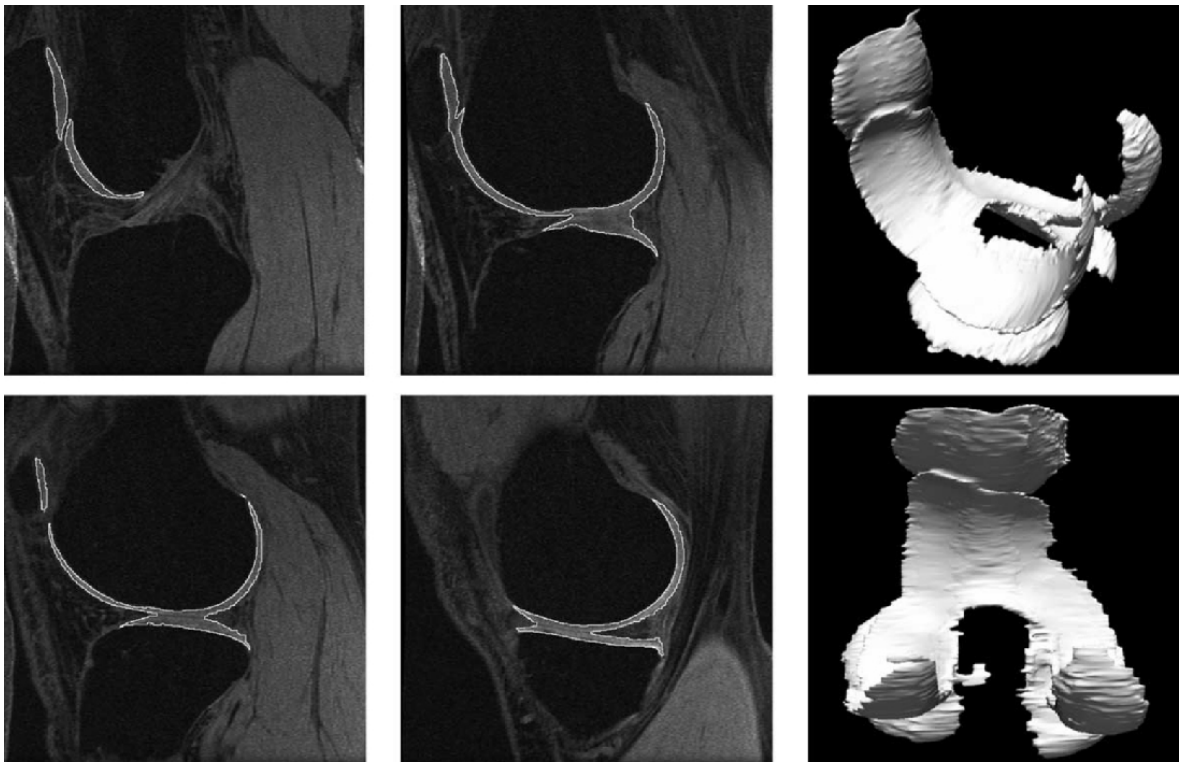


Figure 1.5: Cartilage volume segmentation on MRI.

Cartilage segmentation using improved watershed transform and 3D reconstruction for whole cartilage volume measurement. Source: V. Grau et al. "Improved watershed transform for medical image segmentation using prior information". In: IEEE Trans Med Imaging *23.4 (2004), pp. 447–458*

Higher knee cartilage volume without defects may reflect healthier hyaline cartilage. Cicuttini et al reported that quantitative cartilage volume measured on MRI is considered an accurate method for assessing cartilage health and changes in cartilage volume predict future need for surgical intervention in patients with mild to moderate symptomatic radiographic knee OA [169]. MRI cartilage volume loss is found to have a significantly high correlation with radiographic assessment [170]. Both medial and lateral tibial cartilage volume shows a strongly inverse association with radiographic joint space narrowing [171].

Cartilage volume loss measured by MRI may be associated with knee pain and may predict change in symptoms. Wluka et al reported that symptoms of knee OA, including pain, stiffness and function, are inversely but weakly associated with tibial cartilage volume. Also,

there is a weak association between increased tibial cartilage volume loss and worsening of symptoms in knee OA [172]. Similarly, Raynaud et al found a weak association of cartilage volume loss with simultaneous knee pain change over 24 months [173]. The greatest cartilage volume loss is found in the central area of the medial tibial plateau and of the medial femoral condyle [174].

These studies have also found that cartilage volume loss was predictive of the occurrence of total knee replacement. Cicuttini et al found that 1% increase in tibial cartilage volume loss over two years was associated with a 20% increase in risk for surgery and patients with tibial cartilage volume loss over 8% had a higher risk for surgery than those with less than 3% loss [169]. This result was confirmed by Raynauld and colleagues that medial cartilage volume loss over two years was a strong predictor for total knee replacement over 4 to 7 years [175].

1.3.2 Cartilage defects

In addition to quantitative assessment of cartilage volume, semi-quantitative measurement of focal cartilage defects is another way to evaluate the morphological characteristics of regional articular cartilage. Cartilage defects are localized lesions or tears within the cartilage visible on T1-weighted or T2-weighted MRI (Figure 1.6) [176]. Knee cartilage defects are very common in healthy individuals [177, 178]. Cartilage defects have a relatively variable natural history, but they are less likely to regress in older adults than in younger population [178]. Although the etiology of cartilage defects remains unclear, they are often thought to be related to trauma [179] and could be an important MRI marker of early cartilage damage.

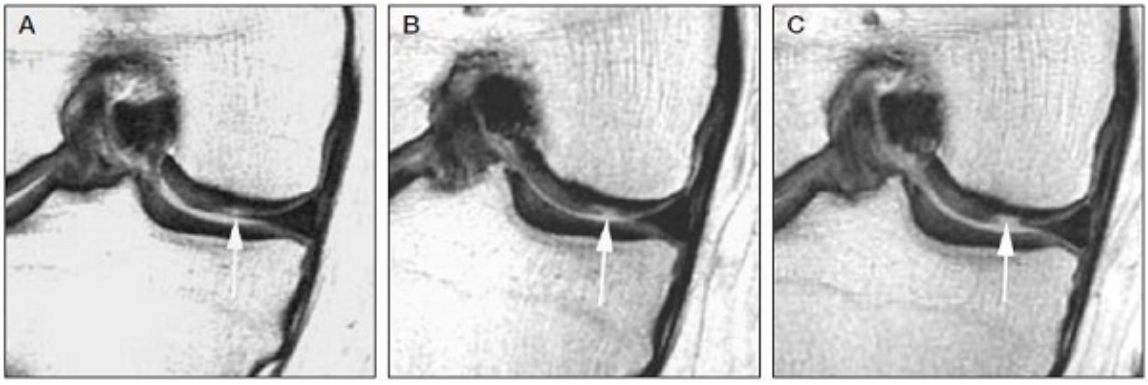


Figure 1.6: Development of a focal cartilage defect over 24 months.

(A) Coronal intermediate-weighted image showing a small superficial cartilage defect in the weight-bearing part of the medial femoral condyle (arrow). (B) Follow-up MRI at 12 months reveals extension of defect to the subchondral bone, now representing a full-thickness focal lesion (arrow). (C) MRI image at 24 months showing a slight increase in the size of the focal full thickness defect. Source: Role of imaging in osteoarthritis: diagnosis, prognosis, and follow-up. http://www.medicographia.com/2013/10/role-of-imaging-in-osteoarthritis-diagnosis-prognosis-and-follow-up/.

Cartilage defects are associated with radiographic features of knee OA measured using Kellgren-Lawrence score [180, 181, 158]. In a study of 224 patients with symptomatic knee OA, the grade of cartilage defects was associated with joint space narrowing [182]. Similarly, osteophyte score has been shown to be correlated with the grade of cartilage defects [158, 183].

The severity of cartilage defects has been shown associated with knee pain in a number of studies [172, 154, 159, 160]. One study found that the grades of cartilage defects were associated with clinical symptoms including pain, stiffness and limited function assessed by WOMAC scores [158]. The correlation between cartilage defects and knee pain has been reported in particular when the defect was moderate to severe (grade 2-3) on a modified Outerbridge scoring system [161, 162], and full-thickness cartilage defects accompanied by subchondral bone exposure were most significantly associated with pain in knee OA [159].

While cartilage defects are not synonymous with cartilage loss, there is a dose-response relationship between cartilage defect score and cartilage volume loss [183]. However, there was only a moderate correlation between histology of grade 1 cartilage defect (abnormal

intrachondral signal) and cartilage breakdown [184]. Cartilage defects are also associated with bone marrow lesions [185] as full-thickness cartilage defects expose the subchondral bone plate to increased loading pressure leading to bone injury.

Cartilage defects are predictive of total knee replacement. One study in a population with established OA showed that higher total cartilage defect scores increased the risk of joint replacement over 4 years by six times compared to those with lower scores [186]. This finding was confirmed in a population-based cohort study where knee cartilage defects were found to independently predict the risk of total knee replacement over 5 years [178].

1.3.3 Bone marrow lesions

Bone marrow lesions (BMLs) on MRI have been recognized as an important feature in knee OA [187, 188]. They are characterized as non-cystic ill-defined subchondral areas of low signal intensity on T1-weighted and high signal intensity on T2-weighted or proton density-weighted fat-suppressed (FS) fast spin echo (FSE) or short tau inversion recovery (STIR) images (Figure 1.7) [189].

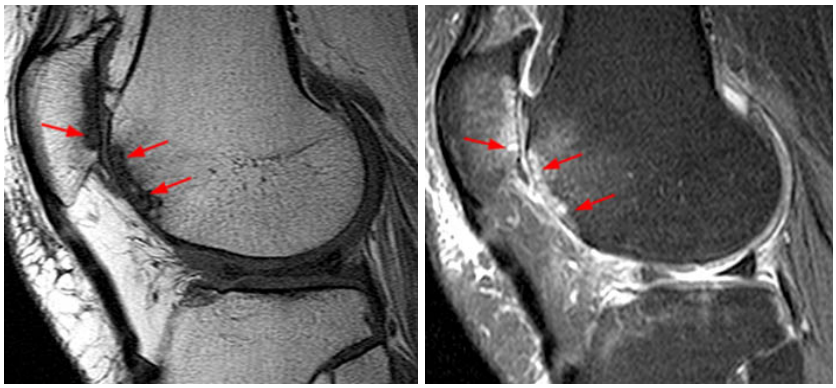


Figure 1.7: Bone marrow lesions on T1-weighted (left) and STIR (right) MRI scans.

Sagittal T1-weighted (left) and STIR (right) images demonstrate bone marrow lesions at the patellofemoral compartment (arrows). Source: Osteoarthritis (OA) of the Knee. Radsource. http://radsource.us/osteoarthritis-oa-of-the-knee/.

Regional bone marrow signal intensity alteration on MRI was initially described by Wilson

et al, using the term 'bone marrow edema' to describe MRI findings in patients with severe knee and hip pain without any specific radiographic abnormalities [190]. Since then, histological studies have shown that various pathologic entities could exhibit the same pattern on MRI, not only edema [191, 192, 193]. Histological study by Zanetti et al found that BMLs in knees in subjects with severe OA undergoing total knee replacement consisted of several abnormalities including bone marrow necrosis (11%), abnormal trabeculae (8%), bone marrow fibrosis (4%), bone marrow edema (4%), and bone marrow bleeding (2%) [194]. Recently, Hunter et al [195] demonstrated that BMLs are sclerotic compared with unaffected regions from the same individual based on the increased bone volume fraction and increased trabecular thickness. As a result, the term 'BML' has become more commonly used in the OA research community [196].

There is increasing evidence linking BMLs to knee pain. In a cross-sectional study in patients with knee OA, Felson et al found that BMLs were associated with the presence of knee pain and large lesions appeared exclusively in those with pain [187]. Similarly, Sowers et al reported that BMLs $>$1 cm were more frequently found in the painful knee OA group than the painless knee OA group [159]. The cross-sectional association between BMLs and pain was confirmed by Zhai et al in a population-based study of older adults [161]. The longitudinal relationship between progression of BMLs and pain was also observed in a number of prospective cohort studies. Data from the Multicenter Osteoarthritis (MOST) Study showed that incidence or progression of BMLs was higher at follow-up in subjects with pain than those without [197] and changes in BMLs were associated with fluctuation in knee pain [198]. Similarly, in a prospective study in community-dwelling older adults, Dore et al found that a change in BML size was associated with changes in pain in those with early stage disease [199], although some studies reported no association between pain and BMLs [200, 201, 158]. A recent systematic review including 9 cohort, 18 cross-sectional and 5 case-control studies concluded that BMLs are independently associated with longitudinally

increasing pain severity and are associated with incident frequent knee pain [202].

It has been suggested that BMLs may be an early MRI biomarker for knee OA and an independent predictor of structural progression in knee OA. Studies have shown that BMLs predict cartilage defect progression [203, 204, 205, 206] and cartilage volume loss [207, 173, 174, 208, 209] on MRI. Increasing or progressive BMLs are also associated with increased cartilage loss [210, 211, 212]. Despite the fact that BMLs predict cartilage damage, it still remains unclear whether BMLs precede, accompany, or follow cartilage damage and volume loss in OA [208].

A handful number of studies have also examined the predictive ability of BMLs for total knee replacement [199, 213, 214, 175]. In one study, Tanamas et al showed that the severity of BMLs was positively associated with the risk of total knee replacement over four years in 109 patients with well-established knee OA [214]. In a recent study by Roemer et al, the risk of total knee replacement was significantly increased in knees with BMLs in more than two subregions [215]. However, most of the studies examined total knee replacement in a severe knee OA sample which has very high rates of total knee replacement, therefore, it is unknown whether BMLs in a community-based sample also predict total knee replacement.

1.3.4 Effusion-synovitis

Synovitis is a common feature of knee OA and occurs in nearly 90% of patients [216]. Because definite synovitis is present in 96.3% knees with effusion [216] and non-enhanced MRI (Figure 1.8) could not readily differentiate synovitis and effusion [217], the term 'effusion-synovitis' indicates that MRI-detected joint effusion equals both inflamed synovium and synovial fluid. Effusion-synovitis could present in different regions within the knee joint, including peri-patellar areas, intercondylar region and around the anterior and posterior cruciate ligament [216].

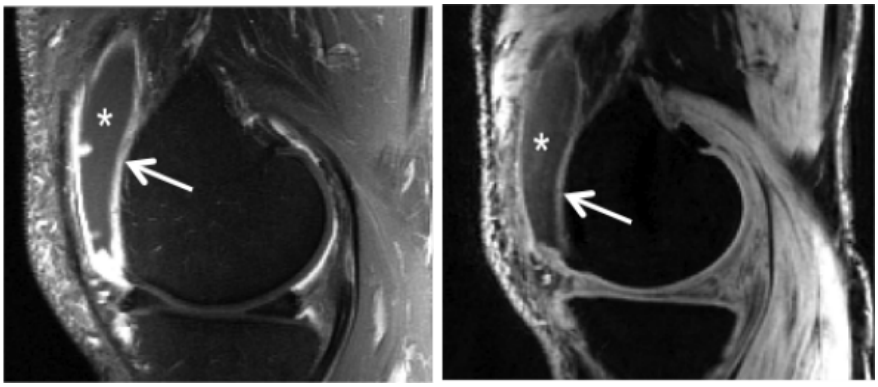


Figure 1.8: Comparison of contrast-enhanced MRI and non-contrast enhanced for synovitis.

Contrast-enhanced T1-weighted (left) and non-contrast DESS (right) demonstrate effusion-synovitis (*) at the suprapatellar pouch. Source: MRI Assessment of Synovitis. http://med.stanford.edu/bmrgroup/Research/musculoskeletal-mri.html.

Effusion-synovitis could occur in early stages of knee OA [218, 219] and is frequently correlated with pain or other clinical outcomes [220, 221]. One study by Torres et al examined the relationship between specific tissue lesion and pain severity in 143 patients with knee OA found that higher grades of effusion-synovitis were significantly associated with the increase in pain severity [160]. A more recent study reported that the association between effusion-synovitis and knee pain may be independent of other structural markers. Effusion-synovitis was more correlated with non-weight-bearing pain, suggesting an important role of inflammation [222]. The association with knee pain was strongest when effusion-synovitis was present within the infra-patellar and supra-patellar areas [223].

It has been suggested that effusion-synovitis in knee OA predisposes to further structural progression [224, 85, 225]. One study using arthroscopy as a reference standard demonstrated a positive correlation between the severity of synovitis and the degree of progression of cartilage lesions over time [86]. Roemer and colleagues examined 514 knees without OA and found that the risk of cartilage loss was significantly increased in knees with effusion-synovitis [85]. More recently, Wang and colleagues reported that effusion-synovitis not only independently predicted the progression of cartilage defects, but also the worsening of BMLs in a general older population over 3 years [225].

There are only a few studies which examined the predictive value of effusion-synovitis for TKR [226]. A large prospective study followed over 500 subjects with knee OA for 3 years demonstrated that the presence of a joint effusion at baseline was a significant predictor of joint replacement at 3 years [227]. A more recent study by Roemer and colleagues reported that the risk of TKR in the subsequent 12 months was significantly increased for knees that exhibited effusion-synovitis at two or more subregions, compared with knees that did not [215].

In summary, MRI has become an important tool to visualize multiple tissues abnormalities in knee OA. Further investigation is needed to examine the predictive validity of these MRI biomarkers for TKR over the long term. Chapter 8 describes the association between these MRI biomarkers and the risk of TKR over 10 years in a general older population.

1.4 Summary

OA is the most common form of arthritis and the knee is the most common joint affected by OA. It is affecting over 1.8 million Australians or 8% of the population. By 2050, it is projected that there will be 3.1 million Australians or 11% of the population with OA. It is a leading cause of disability and costs Australians $1.6 billion of the total health care expenditure in the year 2008-2009.

Currently, there are no approved disease-modifying treatments available for knee OA. There is an urgent need for identifying modifiable risk factors for this disease. Systemic risk factors such as obesity, low-grade systemic inflammation, vitamin D and sex hormones insufficiency may contribute to the disease development through systemic metabolic mechanisms.

It has become clear that knee OA is a disease of the whole joint. MRI is becoming a popular research tool for the visualization of all structures within the knee joint. MRI structural

biomarkers, such as cartilage volume, cartilage defects, BMLs and effusion-synovitis, are found to correlate with disease symptoms and predict the progression of knee OA.

The following chapters investigate the roles of systemic risk factors in disease progression of knee OA and the predictive values of MRI biomarkers for knee replacement, as well as the effects of vitamin D supplementation on the disease. The research questions which directed this work are described in the following chapter.

CHAPTER 2

Research Questions

Chapter 1 reviews the background and rationale of this thesis. The research questions of this thesis are summarized as follows:

1. To determine whether low-grade inflammation plays a role in the pathogenesis of OA by a systematic review of existing evidence.
   (a) Is serum level of high-sensitivity C-reactive protein (hs-CRP) elevated in OA patients compared to control?
   (b) Is serum hs-CRP level correlated with the prevalence or progression of radiographic features of OA?
   (c) Is serum hs-CRP level correlated with the prevalence or progression of clinical symptoms of OA?
2. To describe the longitudinal relationship between adiposity and change in knee pain in a population-based cohort of community-dwelling adults aged 50-80 years examined at baseline and 2.6 and 5 years later.
3. To evaluate the effects of vitamin D supplementation versus placebo on knee pain and knee cartilage volume loss in symptomatic knee OA patients with low vitamin D levels

over 2 years.

4. To describe the longitudinal associations between serum levels of estrogen, progesterone and testosterone and knee structural changes using MRI in both males and females with symptomatic knee OA at baseline and 2 years later.

5. To examine whether cartilage defects, BMLs, effusion-synovitis and meniscal pathologies measured on MRI at baseline predict TKR over 10.7 years in a population-based cohort of community-dwelling adults aged 50-80 years.

   (a) Do semiquantitative MRI measures of cartilage defects, BMLs, effusion-synovitis and meniscal pathologies independently predict the risk of TKR over 10.7 years?

   (b) Does the number of MRI structural pathologies present at baseline predict the occurrence of TKR over 10.7 years?

CHAPTER 3

Methodology

Chapter 4 arose from a systematic review of the literature. Chapter 5 and 8 arose from analyses using the data from the Tasmanian Older Adult Cohort (TASOAC) study. Chapter 6 and 7 arose from analyses using the data from the Vitamin D Effect on Osteoarthritis (VIDEO) study, which is a randomized, double-blind, placebo-controlled clinical trial. This chapter describes the study population and design for the TASOAC and VIDEO study, as well as the protocols for measurement of factors which are common to multiple chapters in this thesis. Additional factors which are unique to each chapter are described in more detail in the methodology section of each of the subsequent chapters.

It should be noted that the following chapters are presented in the form in which they were submitted to, or accepted by, peer-reviewed journals for publication. Thus, throughout these chapters, there are some differences in the description of methods, analyses, results, and interpretations, due chiefly to requests from journal reviewers.

3.1 Study Design of Tasmanian Older Adult Cohort (TASOAC) study

The TASOAC study is an ongoing prospective, population-based study that aims at identifying the environmental, genetic, and biochemical factors associated with the development and progression of OA at multiple sites (hand, knee, hip, and spine).

3.1.1 Study population and design

The cohort consisted of both males and females aged between 50 and 80 years (mean: 62 years; standard deviation (SD): 7 years), selected from the roll of electors in southern Tasmania (population 229,000) using stratified simple random sampling without replacement. Electoral rolls represent the complete population information available in Australia because voting in federal and state elections is compulsory. The sample was stratified by sex to provide equal numbers of men and women, and equal distribution was drawn from urban and rural areas in Southern Tasmania. As TASOAC was designed to examine community-dwelling older adults, institutionalized older adults were excluded. Participants were also excluded if they reported contraindication for MRI (including metal sutures, the presence of shrapnel, iron filings in the eye and claustrophobia), as these tests were required to examine OA progression.

Figure 3.1 provides an overview of participant recruitment and withdrawal during the study period. The first phase of the study was carried out between March 2002 and September 2004. 2,135 initially eligible participants were identified from which 1,904 were able to be contacted. Of all initially eligible participants, 1,100 enrolled in the study, and 1,099 attended a baseline clinic (response rate 51%). The phase 2 follow-up study was conducted 2.6 years later (range 1.4–4.9 years), with a set of measures taken also at a phase 3 follow-up

after 5.1 years (range 3.6–6.9 years), and a phase 4 follow-up after 10.7 years (range 9.2–12.5 years). The MRI machine was decommissioned halfway through the phase 2 follow-up period; therefore, MRI scans were only available for approximately half of the phase 2 follow-up participants. As a result, the sample size used in Chapters 5 and 8 of this thesis varies depending on the available data for each of the research questions.

3.1.2 Sample size

As the TASOAC study was in progress before the commencement of the PhD candidature, formal sample size calculations were not performed during the design of this thesis. Therefore, participant numbers in the analyses reported in this thesis were limited to the numbers recruited at baseline and follow-up, and to those who provided complete data for relevant outcome and study factors. As such, sample sizes vary between chapters, and the reasons for exclusion are described in each chapter. Nevertheless, it subsequently proved that sample sizes were more than adequate to answer the thesis research questions.

3.1.3 Ethical issues

All procedures in TASOAC were approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee (Ethics Approval Number: H6488). Written informed consent was obtained from all participants prior to enrollment in the study.

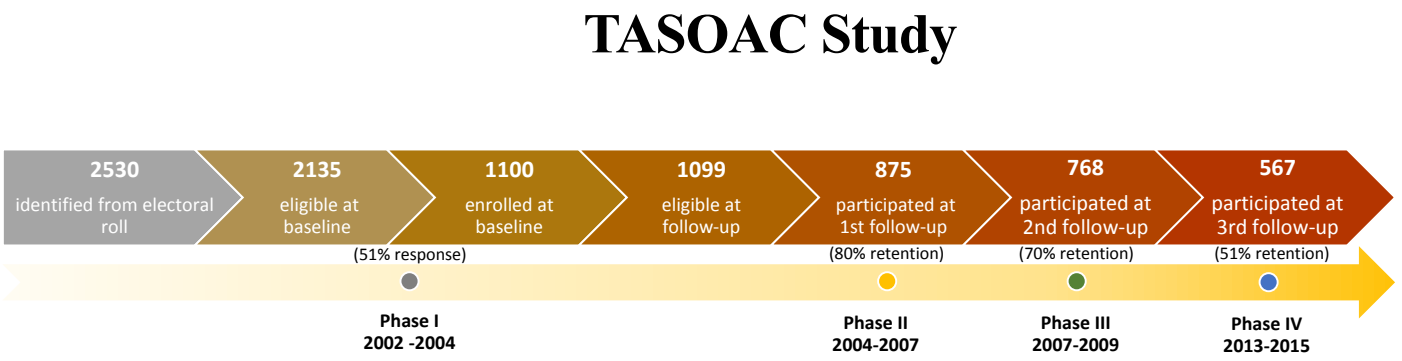


Figure 3.1: Flowchart of TASOAC study participants.

3.2 Study Design of Vitamin D Effect on Osteoarthritis (VIDEO) Study

Vitamin D Effect on Osteoarthritis (VIDEO) study is a randomized, double-blind, placebo-controlled trial with the aim to examine the effects of vitamin D supplementation on knee pain and knee structural changes utilizing pioneering MRI techniques in symptomatic knee OA patients with low serum vitamin D levels.

3.2.1 Study population and design

The VIDEO study recruited patients with symptomatic knee OA in Southern Tasmania and Melbourne, by using a combined strategy, including collaboration with general practitioners, specialist rheumatologists, and orthopaedic surgeons, as well as advertising through local media. The participant inclusion and exclusion criteria for the VIDEO study are listed below:

Inclusion criteria:

1. Age 50-79 years old;
2. Men and women with symptomatic knee OA for at least 6 months with a pain visual analogue scale (VAS) of at least 20 mm;
3. Meet the America College of Rheumatology (ACR) criteria [34] for symptomatic knee OA assessed by a rheumatologist;
4. Have an ACR functional class rating of I, II and III [228];
5. Have relatively good health (0-2 according to the investigators global assessment of disease status on a 5-point Likert scale, range 0 [very well] to 4 [very poor]); and
6. Have serum vitamin D level of $>$12.5 nmol/L and $<$60 nmol/L.

7. Is able to read, speak and understand English, capable of understanding the study requirements and willing to co-operate with the study instructions.

Exclusion criteria:

1. Patients with severe radiographic knee OA (grade 3 according to Altmans atlas [229]);
2. Patients with severe knee pain (on standing more than 80 mm on a 100-mm VAS);
3. Any contra-indication to having an MRI.
4. Patients with rheumatoid arthritis, psoriatic arthritis, lupus, or cancer;
5. Patients with severe cardiac or renal function impairment
6. Patients with hypersensitivity to vitamin D;
7. Patients with any condition possibly affecting oral drug absorption (eg. gastrectomy or clinically significant diabetic gastro-enteropathy);
8. having significant trauma to the knees including arthroscopy or significant injury to ligaments or menisci of the knee within 1 year preceding the study;
9. having anticipated need for knee or hip surgery in the next 2 years;
10. having taken Vitamin D supplements in last 30 days;

Figure 3.2 provides an overview of participant recruitment and withdrawal during the study period. A total of 599 participants were screened for eligibility from 5 Jun 2010 to 1 Dec 2011.

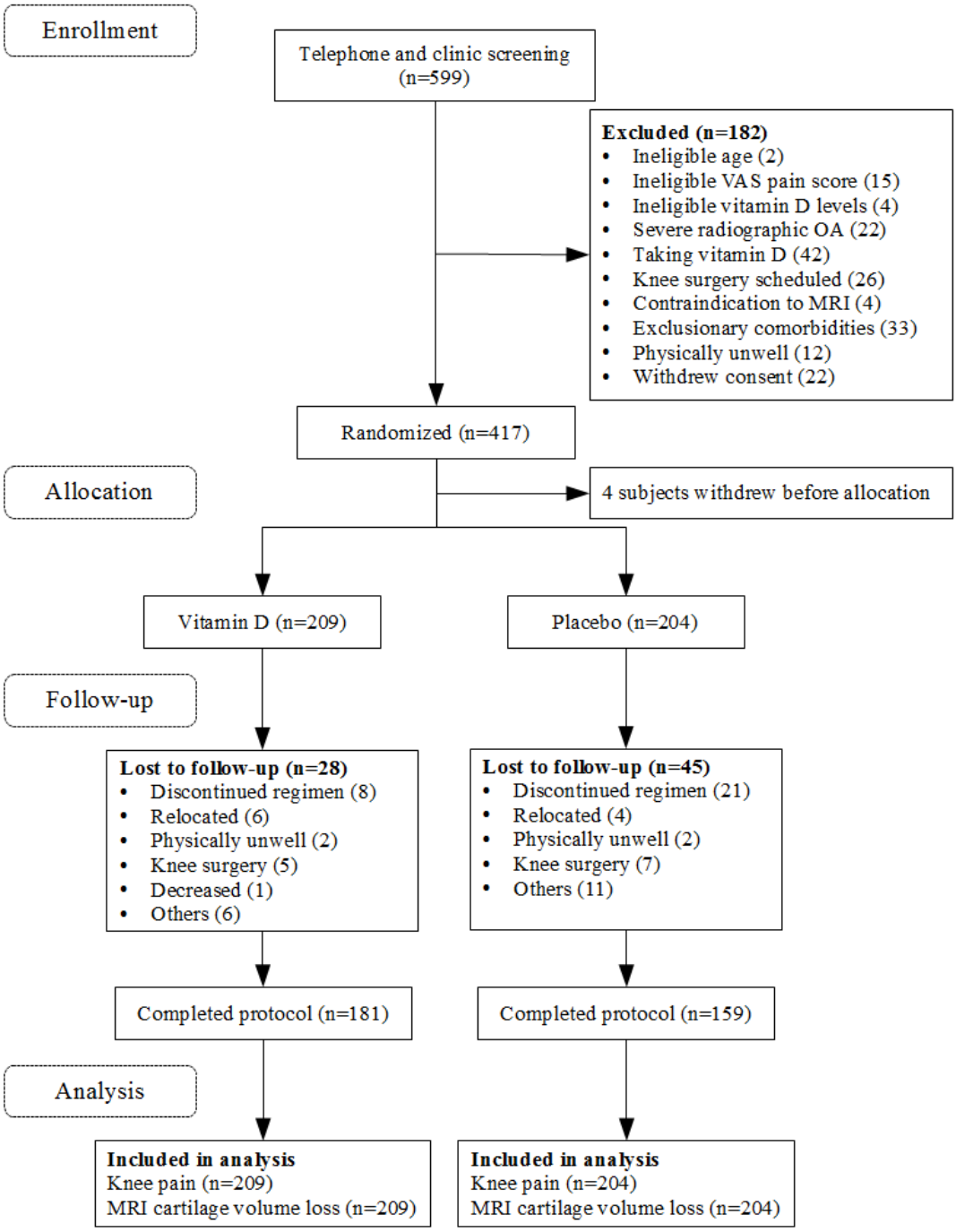


Figure 3.2: Flowchart of VIDEO study participants.

3.2.2 Randomization and masking

Participants were allocated to either vitamin D or placebo at a ratio of 1:1 based on computer-generated random numbers. Allocation concealment was ensured by a central automated allocation procedure that was independent of the investigators. Participants, research coordinators and investigators were all blinded to the treatment assignment. Blinding was maintained until all the data were collected, confirmed for accuracy and cleaned, and statistical analyses were performed.

3.2.3 Interventions

Participants in the treatment group were given a monthly capsule of 50,000 IU (1.25 mg) vitamin D3 (cholecalciferol) for 24 months. The vitamin D3 compound was purchased from Nationwide Compounding Pharmacy, Melbourne, Australia. Participants in the control group received an identical inert placebo provided by the same company.

3.2.4 25OHD assays

Serum 25OHD was assayed at screening, month 3 and 24, utilizing a direct competitive chemiluminescent immunoassay (DiaSorin Inc., Stillwater, Minnesota, USA). The intra-assay and interassay coefficients of variation were 3.2% and 6.0%.

3.2.5 Outcomes

Primary outcome measures were change in knee pain assessed using the Western Ontario and McMaster University Index of OA (WOMAC) score [230] and change in tibial cartilage volume on MRI from baseline to month 24. Secondary outcomes included visual analogue

scale (VAS) knee pain, lower limb muscle strength and other structural changes on MRI. A full list of outcome measures for the VIDEO study is shown in Table 3.1.

Table 3.1: Outcomes and timetable

	Screening	Month(s)				
		0	3	6	12	24
Co-primary outcome measure						
MRI (cartilage volume)		●				●
WOMAC		●	●	●	●	●
Secondary outcome measure						
MRI (other structural changes)		●				●
Lower limb muscle strength		●	●	●	●	●
Visual analogue scale	●	●	●	●	●	●
Other measures						
Core musculature measure		●			●	●
Hand grip strength		●	●	●	●	●
Central and upper arm blood pressure		●		●	●	●
Aortic stiffness		●		●	●	●
Physical activity (IPAQ)		●				●
Body fat		●			●	●
Low foot pain		●	●	●	●	●
Low back pain		●		●	●	●
Depression		●	●	●	●	●
Quality of life		●		●	●	●
Previous knee injury and occupation		●				●
Weight		●	●	●	●	●
Height		●				●
Girth measurements		●			●	●
Knee radiograph	●					
Serum 25-(OH)D	●		●			●
Serum calcium, phosphate, creatinine	●		●			
Sun exposure		●		●	●	●
Cigarette smoking		●				●
Diet (FFQ) and pedometer		●				●
Medications	●	●	●	●	●	●
Pill counts and adverse events		●	●	●	●	●

3.2.6 Sample size

Sample size calculation assumed $\alpha = 0.05$ and $\beta = 0.20$, and was performed based on the Cohen formula [231]. Previous studies reported that mean annual medial tibial cartilage volume loss in knee OA patients was 4.5% [232]. Monthly 50,000 IU vitamin D would achieve serum 25OHD levels above 60 nmol/L [233] and this change was estimated to lead to an absolute reduction in medial tibial cartilage loss of 2.2% annually [120], which was expected to translate into a risk reduction of 44% for total knee replacement over 4 years [169]. 400 participants at baseline (200 in each group), allowing 20% dropouts, would have at least 80% power to detect a 2.2% between-group difference in medial tibial cartilage loss. For change in WOMAC pain, the previous study reported a standard deviation of 70.5 on a score from 0 to 500 [130]. The minimal clinically important difference (MCID) for WOMAC pain was previously reported to be 16% reduction of the score from baseline [234, 235]. With 400 participants a difference between groups of 20 units on the score is detectable with 80% power.

3.2.7 Ethical issues

Ethics approval was received from the Tasmania Health and Human Medical Research Ethics Committee (reference number H1040) and Monash University Human Research Ethics Committee (reference number CF10/1182-2010000616). Informed written consent was obtained from all participants.

3.3 Anthropometrics

Each subjects body weight was measured to the nearest 0.1 kg (with shoes, socks, and bulky clothing removed) using a single pair of electronic scales (Seca Delta Model 707, Bradford,

MA, USA). Height was measured to the nearest 0.1 cm (with shoes and socks removed) using a stadiometer. BMI was calculated as kg/m^2.

3.4 Body composition assessment

Fat mass was measured using a Dual energy X-ray absorptiometry (DEXA) scanner (Hologic Corp, Waltham, Massachusetts, USA). Percentages of total body fat mass and trunk fat mass were calculated as the ratio of total body or trunk fat mass or lean mass by total body or trunk mass (the sum of fat mass, lean mass and bone mass). The coefficients of variation for total body fat, trunk fat mass and lean mass measures were 31.0%, 36.8% and 20.2%, respectively.

3.5 X-ray

A standing anteroposterior semiflexed view of the right knee with 15° of fixed knee flexion was performed. Radiographs were assessed using the atlas developed by Altman et al [229]. Each of the followings was assessed on a scale of 0–3: medial joint space narrowing (JSN), lateral JSN, medial femoral osteophytes, medial tibial osteophytes, lateral femoral osteophytes, and lateral tibial osteophytes. Each score was determined by consensus of two readers who simultaneously assessed the radiograph with immediate reference to the atlas. Intra-observer repeatability was assessed in 40 subjects with an interval of at least one week between the two measurements. Intraclass correlation coefficients (ICCs) ranged from 0.65–0.85. The presence of radiographic osteoarthritis (ROA) was defined as any score ≥ 1 for JSN or osteophytes.

3.6 Magnetic resonance imaging

In the TASOAC study, MRI of the right knee was acquired with a 1.5T whole-body magnetic resonance unit (Picker, Cleveland, OH, USA) using a commercial transmit/receive extremity coil. Image sequences included the following: (1) a T1-weighted fat saturation three-dimensional (3D) gradient-recalled acquisition in the steady state, flip angle 30°, repetition time 31 ms, echo time 6.71 ms, field of view 16 cm, 60 partitions, 512 × 512 pixel matrix, acquisition time 5 min 58 s, one acquisition; sagittal images were obtained at a slice thickness of 1.5 mm without an inter-slice gap; and (2) a T2-weighted fat saturation two-dimensional (2D) fast spin echo, flip angle 90°, repetition time 3067 ms, echo time 112 ms, field of view 16 cm, 15 partitions, 228 × 256 pixel matrix; sagittal images were obtained at a slice thickness of 4 mm with an inter-slice gap of 0.5–1.0 mm.

In the VIDEO study, MRI scans of the study knee were obtained according to a standardized protocol, on a 1.5T whole-body MRI unit using a commercial transmit-receive extremity coil. The sequences used for cartilage volume assessment were sagittal fat saturated (FS) T1-weighted spoiled gradient echo (GRE) with flip angle 30°, repetition time 31 ms, echo time 6.71 ms, field of view (FOV) 160 mm, acquisition time 5 min 58 s, 60 slices, 512 × 512 pixel matrix slice thickness of 1.5 mm without between-slice gap. Cartilage defects and bone marrow lesions were assessed on the T2-weighted/proton density-weighted fast spin echo (FSE) sequences: sagittal FS T2-weighted 3D FSE sequence, flip angle 90°, repetition time 3,067 ms, echo time 112 ms, FOV 16 cm, 15 slices, 228 × 256 pixel matrix slice thickness of 2 mm with a between-slices gap of 0.5–1.0 mm; coronal FS proton density-weighted FSE sequence, repetition time 3,400 ms, echo time 64 ms, flip angle 90°, slice thickness 3 mm, FOV 16 cm, pixel matrix 256 × 256, acquisition time 5 min 26 s. MRIs were assessed by trained readers blinded to treatment allocation.

3.7 Knee pain assessment

Knee pain was assessed on the right knee using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC), a self-administered questionnaire [230]. There are five categories of pain in the questionnaire (walking on flat surface, going up/down stairs, standing upright, in bed when at night, and sitting/lying). In the TASOAC study, each category of WOMAC knee pain was rated on a 10-point numeric scale from 0 (no pain) to 9 (most severe pain). In the VIDEO study, it was measured on a 100 mm visual analogue scale (score range 0–100).

3.8 Statistical analysis

T-tests and chi-squared tests were used to compare differences in means and proportions as appropriate. Standard diagnostic checks of model fit and residuals were routinely performed, and data points with large residuals and/or high influence were investigated for data errors. A p value less than 0.05 (two-tailed) is considered statistically significant. A more detailed description of statistical analyses performed is presented in their relevant chapters. All statistical analyses were performed on Stata for Windows (version 13.0, Stata Corporation, TX, USA).

CHAPTER 4

Circulating C-Reactive Protein in Osteoarthritis: a Systematic Review and Meta-Analysis

4.1 Introduction

Although OA has generally been perceived as a "non-inflammatory "arthropathy, recent studies have suggested that local inflammation plays a prominent role in its pathogenesis [236]. Pro-inflammatory cytokines, including interleukin 1β (IL-1β), tumor necrosis factor-α (TNF-α), IL-6 and others, are produced by synovium and chondrocytes and contribute to the progression of cartilage degradation [237]. A number of studies suggest that this local inflammation may be observed systemically [101, 238, 239, 240].

C-reactive protein (CRP) is an acute-phase protein that is produced by hepatocytes and adipocytes and regulated by pro-inflammatory cytokines [241]. An immunoassay was introduced in 1992 by Montagne and colleagues, to perform high sensitive C-reactive protein (hs-CRP) measurement [242]. In 1997, a study by Loose et al showed that OA patients had higher serum hs-CRP levels than age-matched controls [243]. Since then, numerous studies have been conducted to explore the association between serum CRP levels and OA. While

some studies reported that serum CRP levels were significantly increased in OA [90, 244, 245], others showed no association between hs-CRP levels and OA after the adjustment for body mass [246, 247, 248]. The aim of this study, therefore, was to perform a systematic review of the associations between hs-CRP levels and OA and the individual features of OA.

4.2 Objectives

1. To determine whether hs-CRP levels are elevated in OA patients compared to control;
2. To determine the correlation between serum hs-CRP levels and the following OA features:
    - Prevalence or progression of radiographic features of OA (eg. joint space width, osteophyte, classified Kellgren-Lawrence score, etc).
    - Prevalence or progression of joint symptoms (joint pain, stiffness or physical dysfunction).

4.3 Method

4.3.1 Literature Search

A systematic search on literature from January 1992 to December 2012 was performed on electronic databases including MEDLINE, EMBASE and CINAHL. MeSH terms "osteoarthritis", "inflammation", "c-reactive protein"and related free text terms were used for the search. Search filters designed by Scottish Intercollegiate Guidelines Network (SIGN) for observational studies were incorporated into the electronic database search strategies [249]. Results were then limited to human epidemiological and clinical studies in English. The search strategy for each electronic database is detailed in the Appendix A. We tried to

identify ongoing clinical trials by electronically searching ClinicalTrials.gov, WHO International Clinical Trial Registration Platform Search Portal, and Australian and New Zealand Clinical Trial Registry. The reference lists of obtained studies from the initial electronic search were scanned for further unidentified relevant studies. Conference abstracts from the American College of Rheumatology (ACR) and the European League Against Rheumatism (EULAR) were also searched.

4.3.2 Selection of Studies

Two independent investigators (XJ and JRB) were assigned for the selection of studies. Titles, abstracts, keywords and information of identified studies were entered in an Inclusion/Exclusion form (Appendix A). The initial screening was set to be relatively open-ended to retain as many relevant studies as possible. Full-text was then further examined if the collected information of a primary study suggested that it might meet the inclusion criteria for this review. When information from the published article was not sufficient to make a judgment, correspondent authors were contacted to obtain further information. Discrepancies between two investigators were addressed by consensus after discussion.

Inclusion Criteria

Studies that fulfilled the following criteria were included in this systematic review.

1. Studies included patients with OA;
2. Serum CRP levels were measured using high-sensitivity methodology;
3. Study compared OA patient with healthy subjects, or associated serum hs-CRP levels with phenotypes of OA (e.g. radiological grading, joint space narrowing, pain score and dysfunction score);

4. The article represented original data;

5. Human study;

6. Studies published in English.

4.3.3 Exclusion Criteria

1. Studies included patients with inflammatory joint diseases and other acute inflammatory conditions;

2. Review article;

3. In vitro, animal or ex vivo study.

4.3.4 Data Extraction

One investigator (XJ) extracted the data from included studies using a pre-designed data extraction form (Appendix A). The accuracy of the data was verified by a second investigator (JRB). Study characteristics were recorded including publication information, study design, origin of study, study setting, time frame of study, age, gender split, BMI, definition of OA, affected joints, hs-CRP measuring method, serum hs-CRP levels, relative measures and correlational data with OA.

4.3.5 Quality Assessment

Two investigators (XJ and JRB) independently evaluated the methodological quality of all included studies. The assessment was based on the Newcastle-Ottawa Quality Assessment Scale (NOS) for Case-Control Studies [250] with modifications to accommodate the topic of this review (Appendix A). The NOS was identified to be one of the two useful tools to assess

the quality of non-randomized studies in a systematic review of 182 tools [251]. The total quality score was not utilized in the meta-analyses, as we believed it was more appropriate to assess different aspects of methodological quality of a study in a separate manner.

4.3.6 Assessment of Heterogeneity

Heterogeneity across included studies was examined using Cochran Q test and I^2 test. A result of Chi^2 >25% and p <0.10 was defined as evidence of significant heterogeneity across studies. To further analyze heterogeneity, the I^2 test was used to estimate the extent of heterogeneity, for example, the percentage of variation across studies that is not caused by chance. A I^2 value higher than 30% would indicate moderate heterogeneity and a value higher than 50% would represent substantial heterogeneity [252]. Possible sources of heterogeneity and their effects on the results were explored by subgroup analyses and sensitivity analyses.

4.3.7 Assessment of Publication Bias

Publication bias and "small-study effects" were evaluated using a funnel plot. Asymmetry identified on the funnel plot would imply possible publication bias. A modified Eggers regression test was performed to detect the publication bias. When p-value equaled or was less than 0.10, significant publication bias was considered [253].

4.3.8 Data Synthesis and Analysis

For dichotomous data, a pooled odd ratio (OR) and a 95% confidence interval (95% CI) were computed by the Mantel-Haenszel method. For studies that reported relative risks (RR), we made an attempt to reconstruct a 2×2 table with information provided in the text and calculated the OR.

Because crude hs-CRP levels are frequently skewed, some individual studies had normalized the hs-CRP by logarithmic transformation in the statistical analysis while others reported the results on the original scale. In order to allow meta-analysis to be conducted on a common scale, we adopted the methods proposed by Higgins et al to transform data from a logarithmic scale to a raw scale [254]. The unit of hs-CRP measurement was uniformly converted to mg/l in the meta-analysis. The difference in means and its 95% CI were calculated to estimate the difference in hs-CRP levels between OA patients and healthy controls. Generic inverse variance method on the random-effects model was used for the statistical pooling as we expected the true effects would vary across individual studies.

For correlational data, we obtained correlation coefficients (r) and calculated the corresponding standard error by computing the square root of sample variance as below.

$$SE = \sqrt{\frac{1-r^2}{n-2}} \tag{4.1}$$

Correlation coefficients were combined using generic inverse variance method and the random-effects model.

When data were sufficient and appropriate, pre-specified subgroup analyses stratified by age, BMI, joints of OA, definition of OA, hs-CRP measuring methods and study designs were performed to assess the influence of the above parameters. Meta-regression analysis was performed to assess the influence of age, BMI and female sex using the random-effects model.

Statistical analyses were performed using Revman version 5.2 (The Nordic Cochrane Centre, The Cochrane Collaboration, 2012) and STATA (Release 12. College Station, TX. StataCorp LP). All reported p-values were two-sided and $p < 0.05$ was considered to be statistically significant.

4.4 Results

4.4.1 Literature Search

1,198 records were retrieved from the initial search on electronic databases, with 523 from MEDLINE, 346 from EMBASE, and 329 from CINAHL. Twenty relevant abstracts were identified from ACR and 40 from EULAR. Reference mining identified another 14 articles. A total of 1,272 records were identified. A total of 185 duplicated records were removed. After title and abstract screening, 1,025 articles were excluded. Sixty-seven full texts were examined for the eligibility of inclusion. Thirty-five articles were further excluded because they were ex-vivo studies, in-vitro study, review articles, and studies in which OA was not the disease of interest, or CRP levels were not measured, or analysis data on CRP were not performed or not reported in the article. Two articles [239, 255] were also excluded because same data were reported in another article. One study [256], which used the number of OA-affected knees instead of the number of OA patients in the analysis, was excluded. Finally, thirty-two studies met the inclusion criteria (Figure 4.1).

4.4.2 Included Studies

Ten case-control [257, 258, 259, 260, 261, 262, 263, 264, 265, 266], 15 cross-sectional [248, 267, 268, 269, 270, 271, 272, 258, 273, 274, 275, 89, 276, 245], 4 longitudinal studies [277, 278, 279, 280] and 3 clinical trials were included [243, 281, 282]. The three clinical trials had data comparing CRP levels in OA patients with healthy controls at baseline; therefore, their data were included and were assessed as a case-control design. The characteristics of included studies are summarized in Table 4.1. The 32 included studies provided data of 17,090 participants (6,440 OA cases and 10,650 controls) in 12 countries.

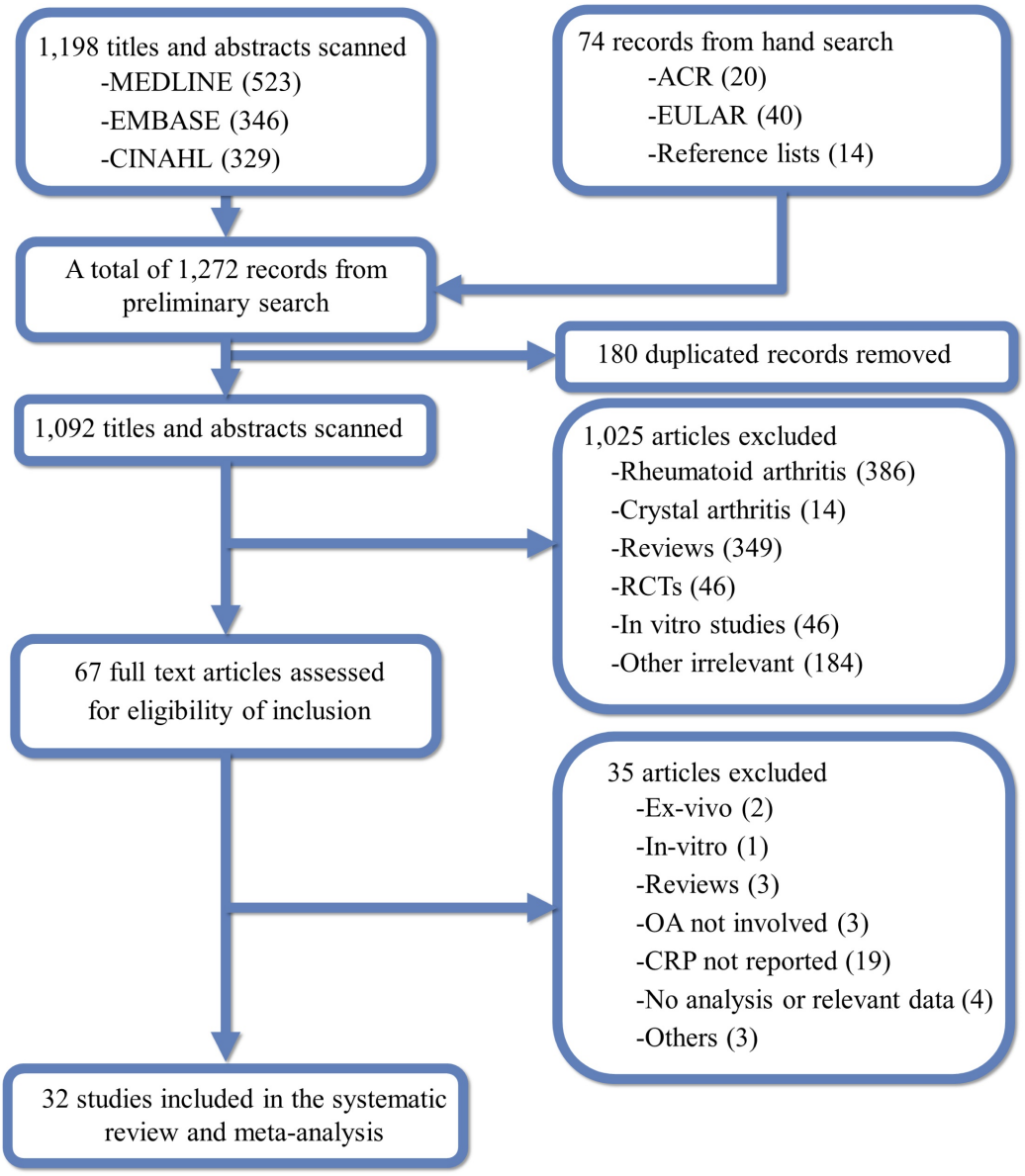


Figure 4.1: Flowchart of Study Selection.

Table 4.1: Characteristics of included studies

Characteristics	Longitudinal	Cross-sectional	Case-control	Clinical-trial	Total
No. of studies	4	15	10	3	32
No. of subjects	1461	13093	2002	534	17090
Mean age (range)	64.9 (62.1–69.1)	60.5 (42.9–71.9)	59.2 (51.1–68.5)	61.1 (59.0–61.9)	61.4 (42.9–71.9)
Female (%)	62.8	38.4	38.2	68.8	52.0
Mean BMI (range)	27.3 (25.7–28.6)	28.12 (19.9–60.45)	27.2 (19.1–46.5)	27.7 (24.3–32.6)	27.6 (19.1–60.45)
Single-centre (%)	25	46.7	90	100	62.5
OA definition* (%)					
Clinical	1 (25.0)	6 (40.0)	5 (50.0)	1 (33.3)	13 (40.6)
Radiographic	3 (75.0)	4 (26.7)	4 (40.0)	0 (0)	11 (34.4)
Both	0 (0)	4 (26.7)	1 (10.0)	0 (0)	5 (16.6)
Registry data	0 (0)	1 (6.6)	0 (0)	2 (66.7)	3 (9.4)
OA sites					
Knee	2 (50.0)	4 (26.7)	5 (50.0)	2 (66.7)	13 (40.6)
Hip	1 (25.0)	3 (20.0)	1 (10.0)	0 (0)	5 (15.6)
Hand	0 (0)	1 (6.6)	2 (20.0)	0 (0)	3 (9.4)
Not specified	1 (25.0)	7 (46.7)	2 (20.0)	1 (33.3)	11 (34.4)
CRP measurement					
ELISA	1 (25.0)	6 (40.0)	4 (40.0)	3 (100)	14 (43.8)
Nephelometry	3 (75.0)	9 (60.0)	6 (60.0)	0 (0)	18 (56.2)

**Clinical, clinically defined OA—for example, ACR criteria; radiographic, radiographically defined OA — for example, KL $\geq$ 2; both, clinically and radiographically defined OA, including knee or hip replacements. ACR, American College of Rheumatology; BMI, body mass index; KL, Kellgren-Lawrence; OA, osteoarthritis.*

Table 4.2: Reasons for excluded studies

Study ID	Reasons for exclusion
Agarwal 2009	OA was not the disease of interest
Attur 2011	CRP was not measured
Attur 2012	CRP was not measured
Botha 2008	CRP was not measured
Calguneri 2003	CRP was not measured
Ding 2009	CRP was not reported
Fonseca 2009	CRP was not measured
Fraenkel 1998	Ex vivo study
Garnero 2006	Review (abstract)
Goekoop 2010	Ex vivo study
Golightly 2011	The number of knees was used as sample size
Gomes 2012	CRP was not measured
Jorgensen 2012	No major observation
Keyszer 1999	OA was not the disease of interest
Ling 2009	CRP was not measured
Massicotte 2002	In vitro study
Meliconi 2006	CRP was not measured
Miller 2008	No comparison between OA and healthy control
Naguib 2010	CRP data were not reported (conference abstract only)
Neidhart 2000	No OA subjects were involved
Otterness 1995	CRP data are reported in another related paper (Otterness 2000)
Otterness 2001	CRP data are reported in another related paper (Otterness 2000)
Pantsulaia 2010	CRP was not measured
Richette 2008	CRP was not measured
Richette 2011	No correlational analysis between CRP and severity of OA
Riyazi 2004	CRP was not measured (conference abstract only)
Romero 2006	CRP was not measured
Scanzello 2009	Commentary review
Sipe 1995	Review
Smith 2012	No available data
Stannus 2010a	CRP was not measured
Stannus 2010b	CRP was not reported
Tian 2011	CRP was not measured
Trontzas 1998	CRP was not measured
Uson 1997	CRP was not measured

4.4.3 Excluded Studies

Excluded studies after full text assessment and reasons for exclusion are listed in Table 4.2.

Table 4.3: Summary of methodological quality of included studies

Study ID	Selection				Comparability		Exposure			Total
	S1	S2	S3	S4	C1	C2	E1	E2	E3	(out of 9)
Bos 2008	•	•	•	•	•				•	6
Brenner 2003	•				•	•	•	•	•	6
Chen 2008	•	•	•	•	•	•	•	•	•	9
Conronzier 1998	•	•	•	•	•	•	•	•	•	9
Conronzier 2000	•		•	•			•	•	•	6
Engstrom 2009	•	•	•	•	•	•	•	•	•	9
Garnero 2005	•	•			•	•	•			5
Garnero 2001	•	•	•	•	•	•	•	•	•	9
Gungen 2012	•	•	•	•	•	•	•	•	•	9
Hulejova 2007	•	•	•	•			•	•	•	7
Hussein 2008	•	•		•			•	•	•	6
Keenan 2008		•					•			2
Kerkhof 2010	•	•	•	•	•	•	•	•	•	9
Kraus 2007	•	•	•	•	•	•	•	•	•	9
Livshits 2009	•	•	•	•	•	•	•	•	•	9
Lee 2011	•	•	•	•	•	•	•	•	•	9
Mazieres 2006	•	•			•	•	•	•	•	7
Melikglu 2009	•			•	•		•	•	•	6
Meulenbelt 2007	•	•			•	•	•			5
Otterness 2000	•			•	•		•	•	•	6
Pearle 2007	•	•	•	•	•	•	•	•	•	9
Punzi 2005	•		•	•	•	•	•	•	•	8
Sharif 1997	•		•	•	•		•	•	•	7
Sharif 2000	•	•					•			3
So 2003	•	•		•			•	•	•	6
Spector 1997	•	•	•	•	•	•	•	•	•	9
Sower 2002	•	•	•	•	•	•	•	•	•	9
Sturmer 2004	•	•	•	•	•	•	•	•	•	9
Takahashi 2004	•	•		•			•		•	5
Vlad 2011	•	•	•	•	•	•	•	•	•	9
Wolfe 1997	•	•			•	•	•	•	•	7
Loose 1996					•		•	•	•	4

4.4.4 Quality Assessment

The results of methodological quality assessment for each individual study were summarized in Table 4.3. Discrepancies between two reviewers were resolved by consensus after discussion. The average methodological quality across included studies was satisfactory. Seventeen studies were scored above six out of nine. However, two studies, Keenan 2008 [274] and Sharif 2000 [278], were graded 2 and 3 respectively, suggesting a higher risk of bias. Another study [243] was only published in abstract and scored in only four items due to limited information. All studies but two [243, 274] provided clear definitions of OA and description of CRP measurement.

4.4.5 Meta-Analyses

The results of all meta-analyses are summarized in Table 4.4.

Table 4.4: Summary of results of meta-analyses

Outcomes	No. studies (No. participants)	Summary measure	95% CI	P value
Serum hs-CRP levels and OA				
Absolute difference (mg/l) (OA vs control)	14 (5,483)	MD = 1.19	0.64 – 1.73	<0.001*
Odds ratio (progressive vs non-progressive)	4 (10,619)	OR = 0.99	0.81 – 1.21	0.93
Correlation between hs-CRP levels and OA phenotypes				
Kellgren-Lawrence grade	2 (95)	r = 0.13	-0.08 – 0.35	0.22
Joint space width	2 (205)	r = -0.10	-0.29 – 0.10	0.33
Pain	5 (1,233)	r = 0.14	0.09 – 0.20	<0.001*
Physical function reduction	2 (244)	r = 0.25	0.13 – 0.39	<0.001*

OA, osteoarthritis; hs-CRP, high sensitivity c-reactive protein.
MD, mean difference; OR, odds ratio; r, coefficient correlation.
** indicates statistically significance.*

4.4.6 Comparison of hs-CRP levels between OA and non-OA

We made efforts to retain all available data from included studies in the meta-analysis. Correspondent authors were contacted by email for studies that had missing data or insufficient information. Eventually, data from 15 primary studies were available for this component of the meta-analysis. Two studies [269, 270] reported hs-CRP levels in different OA subgroups; therefore, we separately entered the subgroup data in the meta-analysis. Six studies [243, 257, 262, 272, 90, 89] had either missing data or insufficient information for transforming data that allow being pooled in the meta-analysis. Data from one study [277] were also not included because observations in the control group were not completely free of OA. A summary of results from these seven studies is presented in Appendix A.

Fifteen studies were included in the meta-analysis of the difference in means of hs-CRP levels between OA patients and healthy controls (Figure 4.2). Except for one study [261] showing no difference between OA and controls, all other studies revealed that circulating levels of hs-CRP were higher in OA patient than in healthy controls. The pooled mean difference showed that hs-CRP level was significantly higher in OA than in controls, with an average increase in value of 1.19 mg/l (95% CI 0.64 to 1.73, $p < 0.001$). There was significant variation across the included studies in terms of the mean difference (MD) of hs-CRP levels, which ranged from 0.06 mg/l to 3.43 mg/l. The test for heterogeneity was statistically significant ($Chi^2 = 76.23$, $p < 0.001$; $I^2 = 79\%$), indicating substantial inter-study variation. Possible explanations for the variation were explored in subgroup analyses and sensitivity analyses.

Publication bias was examined by the evaluation of the symmetry of a funnel plot (Figure 4.3) as well as the Eggers test. The funnel plot suggested that there were potential publication bias and small-study effects in the results. The existence of ”small study effects” was confirmed by the Eggers test. Therefore, we subsequently performed a sensitivity analysis

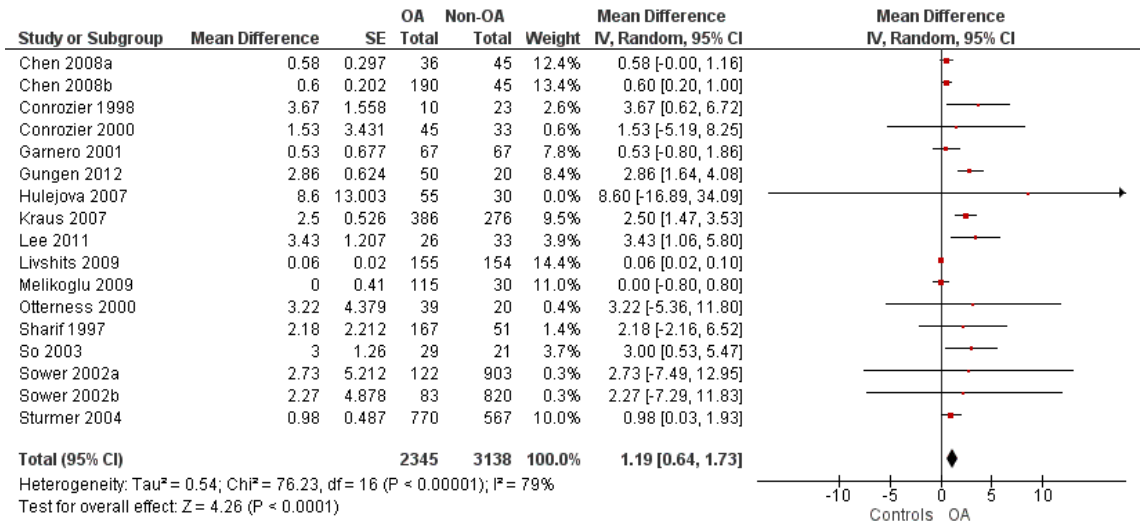


Figure 4.2: Comparison of CRP levels between OA and non-OA.

to examine the impacts of small studies.

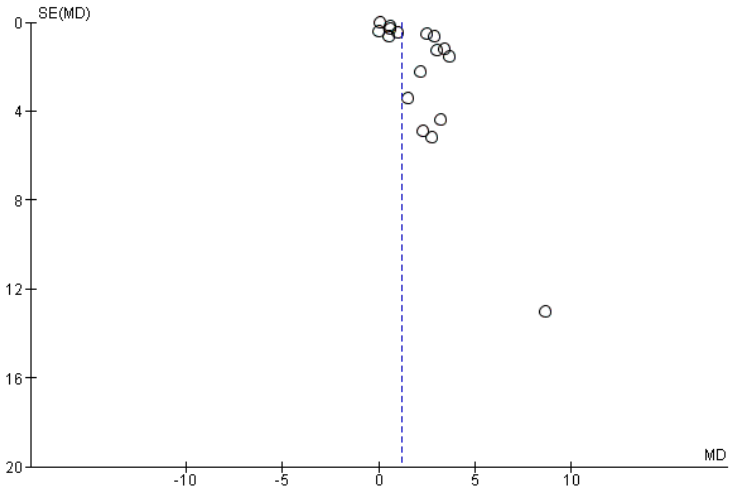


Figure 4.3: Funnel plot of included studies.

4.4.7 Subgroup and sensitivity analyses

Subgroup analyses based on OA joint sites (hip and knee) and diagnosis (clinical and radiological) were performed. Sensitivity analyses based on CRP measurement techniques

(ELISA and nephelometry) (Figure 4.4), study size (small and large) (Figure 4.5), study design (case-control and cross-section) (Figure 4.6) and adjustment for BMI were performed (Figure 4.7). A summary of results is presented in Table 4.5. The increase in hs-CRP level was more considerable in hip OA than in knee OA (3.37mg/l vs 1.15mg/l). Elevated hs-CRP levels were observed both in clinical OA and radiographic OA, 1.76mg/l and 1.07mgl higher than non-OA, respectively. Compared to nephelometry, high sensitivity ELISA was the more sensitive measuring technique. Small studies (N <100) were more likely to report significant differences. The increase in hs-CRP was reported to be more moderate in a case-control design than in a cross sectional design (0.91mg/l vs 1.82mg/l). Control for BMI in a study or adjustment for BMI in an analysis did not significantly change the increase of hs-CRP in OA.

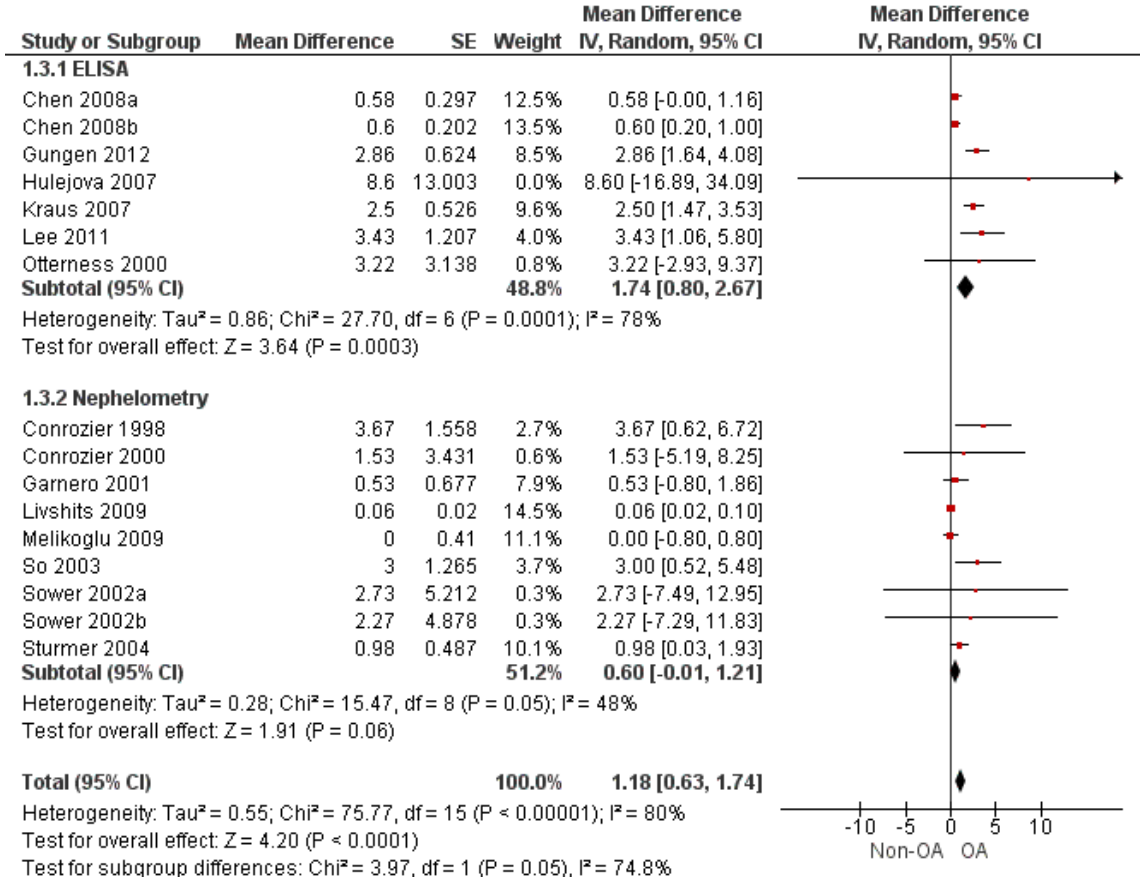


Figure 4.4: Sensitivity analysis: hs-CRP measurement techniques.

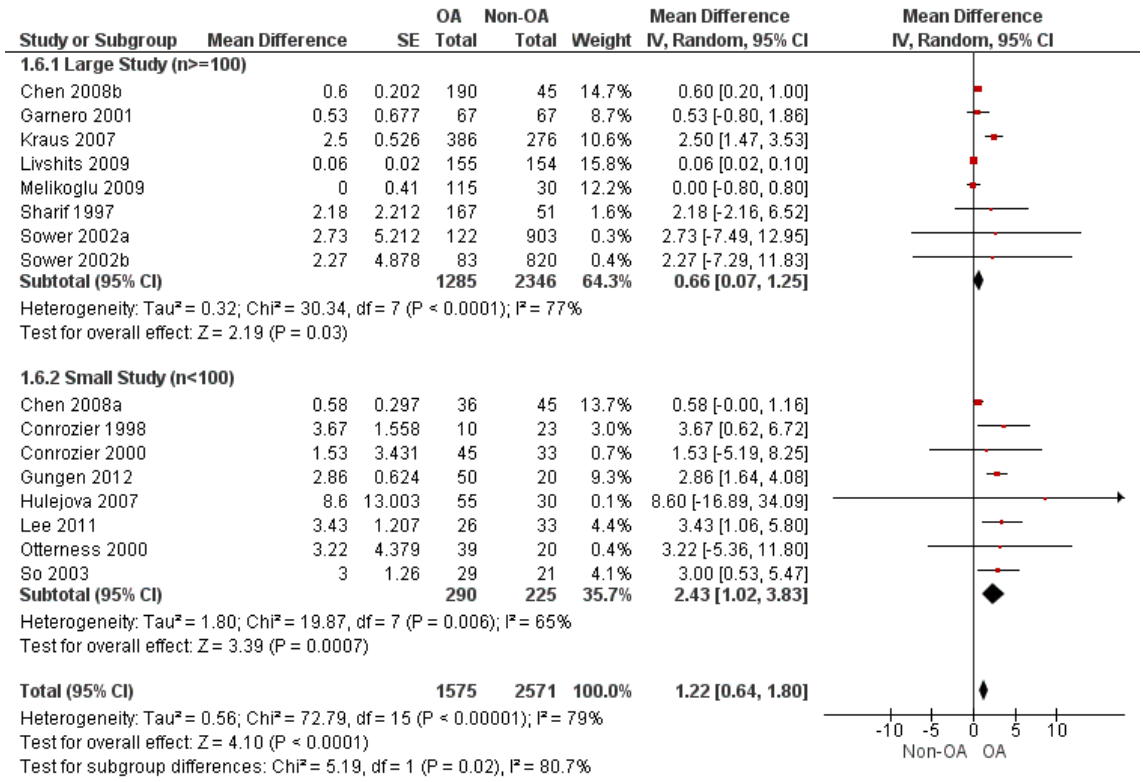


Figure 4.5: Sensitivity analysis: large studies versus small studies.

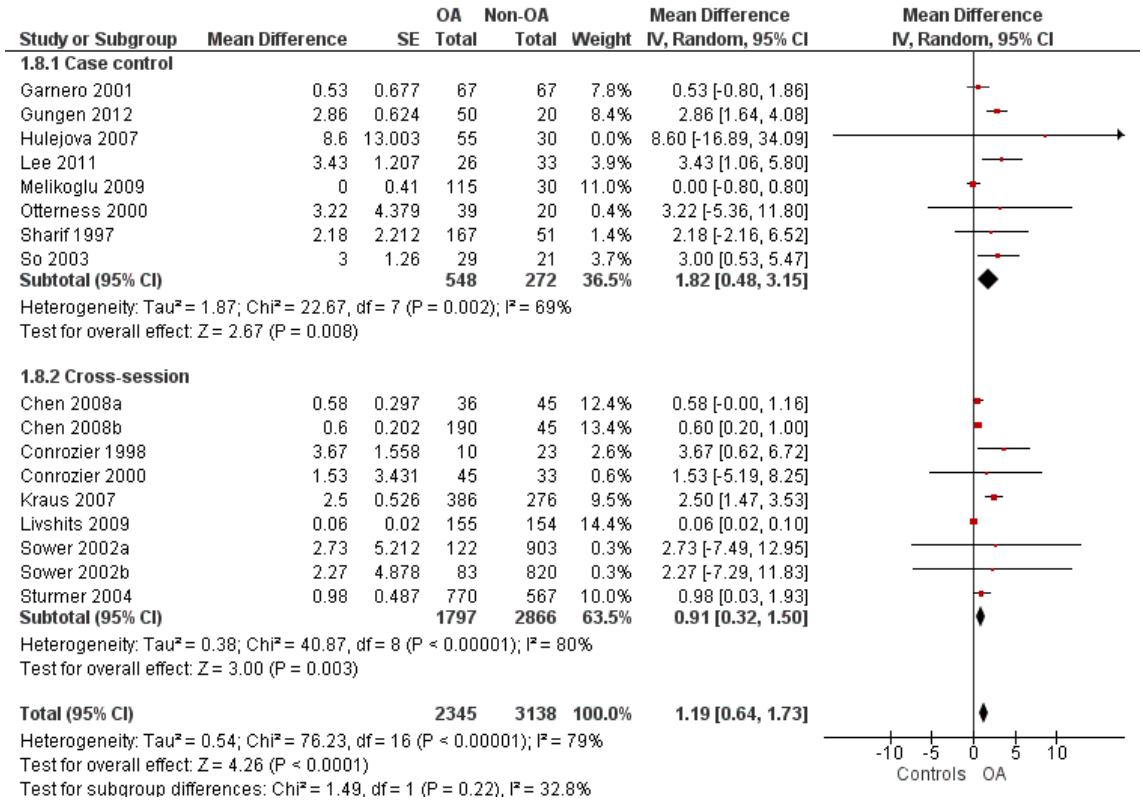


Figure 4.6: Sensitivity analysis: case-control studies versus cross-sectional studies.

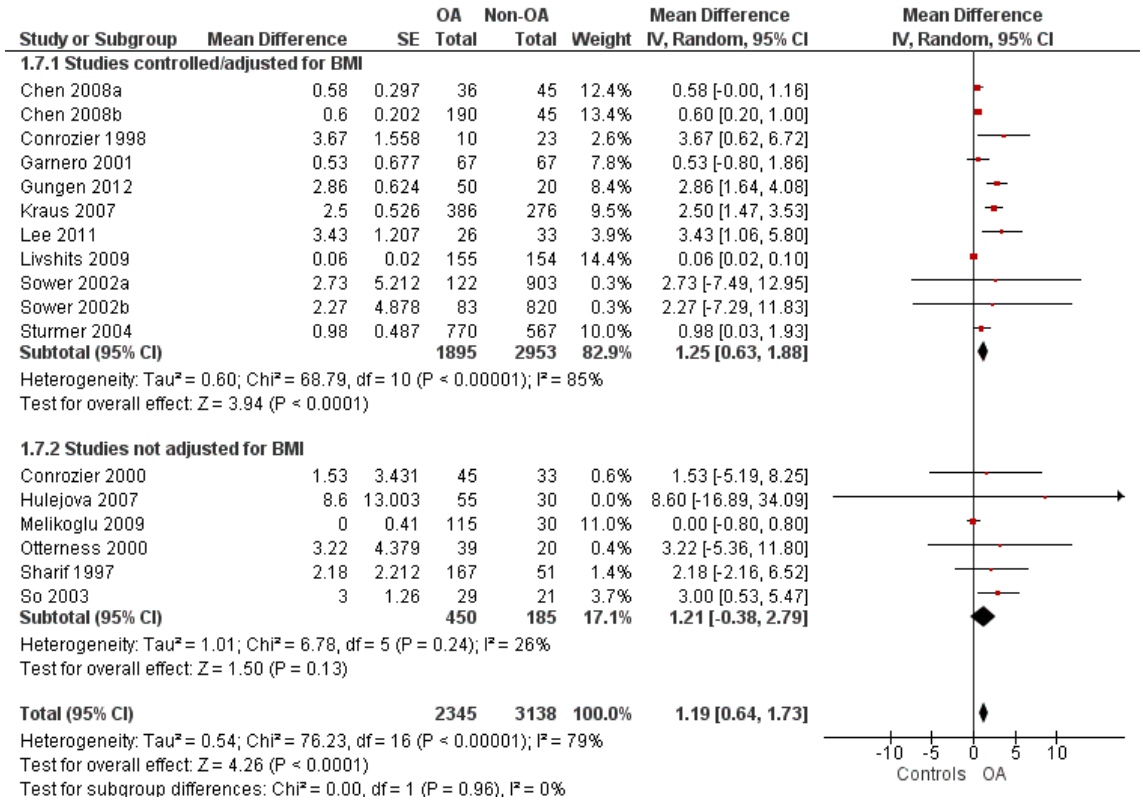


Figure 4.7: Sensitivity analysis: adjustment for BMI versus no adjustment.

Table 4.5: Summary of subgroup and sensitivity analyses of difference in hs-CRP between OA and non-OA

Outcomes	No. studies (No. participants)	Mean difference (mg/l)	95% CI	P value
Joints of OA				
Knee	8 (2,922)	1.15	0.18–2.12	0.02*
Hip	3 (196)	3.37	0.60–6.13	0.02*
Hand	1 (81)	0.58	0.00–1.16	0.05
Not specified	5 (2,343)	1.51	0.49–2.53	<0.01*
Diagnosis of OA				
Clinical OA	10 (2,316)	1.07	0.47–1.66	<0.01*
Radiographic OA	5 (3,167)	1.76	-0.04–3.57	0.05*
Measuring techniques				
ELISA	6 (1,251)	1.74	0.80–2.67	<0.01*
Nephelometry	8 (2,677)	0.6	-0.01–1.21	0.06
Study size				
N $\geq$ 100	8 (4,968)§	0.7	0.16–1.25	0.01*
N <100	8 (515)§	2.43	1.02–3.83	<0.01*
BMI controlled/adjusted				
Yes	9 (4,848)	1.25	0.63–1.88	<0.01*
No	6 (635)	1.21	-0.38–2.79	0.13
Study design				
Cross-section	8 (820)	1.82	0.48–3.15	<0.01*
Case-control	7 (4,663)	0.91	0.32–1.73	<0.01*

OA, osteoarthritis; hs-CRP, high sensitivity c-reactive protein.
§Comparative analyses from one study (Chen 2008) are grouped separately according to their sample size.
** indicates statistically significance.*

4.4.8 CRP levels and OA progression

Three studies reported relative measures, either RR or OR (Figure 4.8), on the predictive value of hs-CRP levels for progression of OA as either exacerbation of joint space narrowing or total joint replacement. The pooled OR showed no significant predictive value of hs-CRP level in the progression of OA (OR = 0.99, 95% CI 0.81 to 1.21, p = 0.93). The test for heterogeneity was borderline significant (Chi^2 = 7.02, p = 0.07; I^2 = 57%).

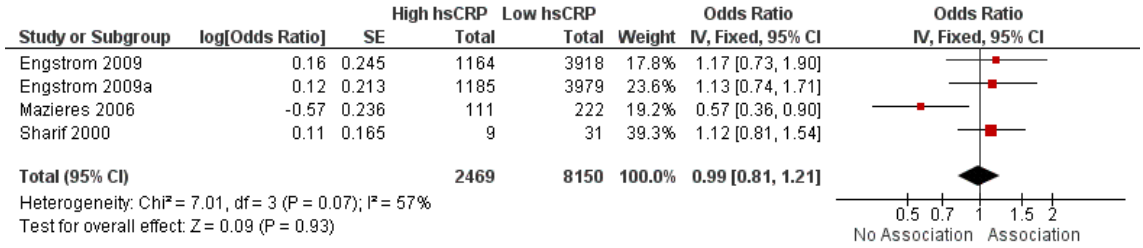


Figure 4.8: Serum hs-CRP levels and progression of OA.

4.4.9 Correlations between hs-CRP levels and OA phenotypes

hs-CRP and radiographic OA

The links between serum hs-CRP levels and knee radiographic OA were investigated in four studies (Figure 4.9). The pooled correlation coefficient showed that the link between serum hs-CRP levels and knee radiographic OA was weak and it was not statistically significant (r = 0.11, 95% CI -0.03 to 0.26, p = 0.13). The correlation coefficients remained non-significant when we separated studies into Kellgren-Lawrence grading subgroup (r = 0.13, 95% CI -0.08 to 0.35, p = 0.22) and joint space narrowing subgroup (r = 0.10, 95% CI -0.10 to 0.29, p = 0.33). There was no significant difference between the two subgroups (Chi^2 = 0.07, p = 0.79; I^2 = 0%).

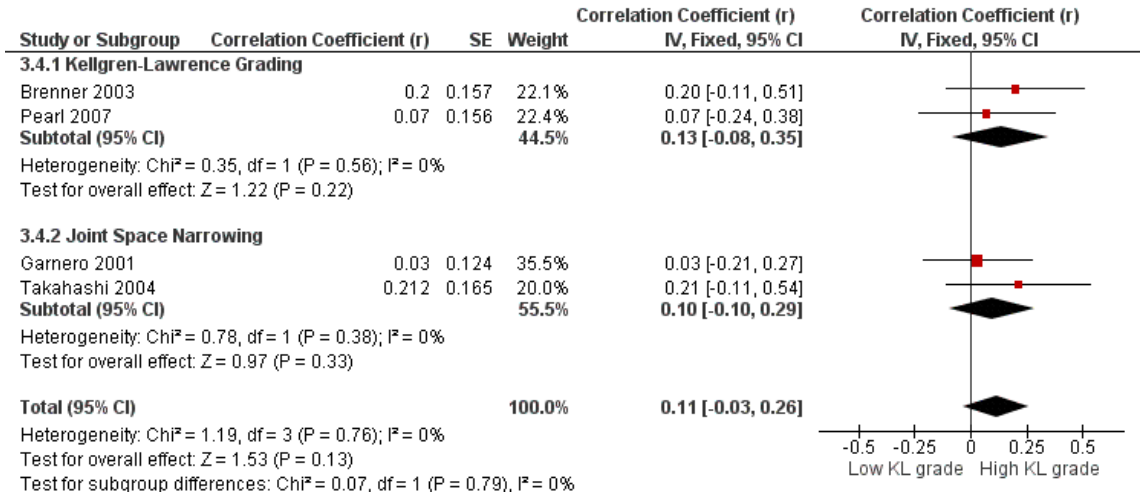


Figure 4.9: Correlation between hs-CRP levels and radiographic OA.

hs-CRP and symptoms of OA

The correlation coefficients between hs-CRP levels and pain in OA patients were available in six studies. A total of 840 OA patients were included in the meta-analysis (Figure 4.10). Brenner study reported a negative correlation between hs-CRP levels and VAS pain score, while the other four all reported a positive correlation. The pooled result of the meta-analysis showed that there was a weak but statistically significant correlation between hs-CRP levels and pain scale score (r = 0.14, 95% CI 0.08 to 0.20, p <0.001). There was no significant heterogeneity observed across the studies (Chi^2 = 4.12, p = 0.39; I^2 = 3%).

Two studies reported the correlation coefficient of hs-CRP with physical function (Figure 4.10) [262, 276] . The results from both studies were consistent with each other, indicating a correlation between increased hs-CRP levels and worsening physical function. The pooled correlation coefficient was statistically significant (r = 0.26, 95% CI 0.13 to 0.39, p <0.001).

The pooled results of correlation data for symptoms of OA were statically significant (r = 0.16, 95% CI 0.11 to 0.22, p <0.001). There was no significant difference between correlation with pain and correlation with physical function loss (Chi^2 = 2.48, p = 0.12; I^2 =

59.7%).

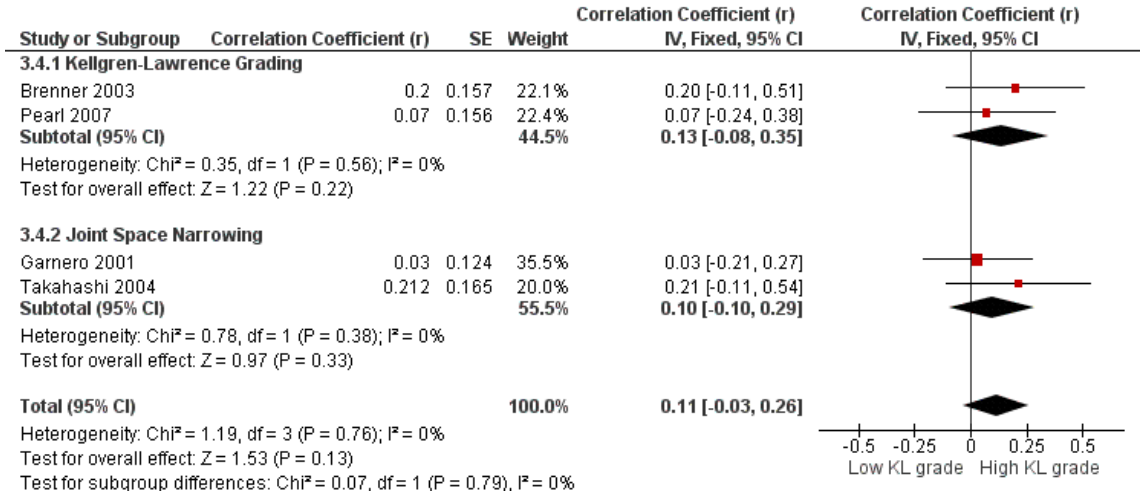


Figure 4.10: Correlation between hs-CRP levels and symptoms of OA.

4.5 Discussion

This study is a comprehensive systematic review of serum hs-CRP levels in OA. A related meta-analysis of three studies previously performed in 2010 by Kerkhof et al did not provide evidence of an association between serum CRP levels and knee OA [277]. In the present systematic review, we included all available data in our meta-analysis on the difference of serum hs-CRP levels between OA population and healthy controls. In addition, the association between hs-CRP levels and different OA phenotypes was also examined.

The major findings of this systematic review are: 1) serum hs-CRP level is modestly elevated in the OA population, as reported in most studies (19 out of 21). (MD = 1.19 mg/l, $p < 0.001$); 2) serum hs-CRP level is associated with symptoms of OA, such as pain and loss of physical function,; 3) serum hs-CRP level is not significantly associated with KL scores and joint space narrowing.

The first result above should be regarded with caution due to the significant heterogeneity.

We identified some reasons for this with the apparent difference being greater in studies using ELISA as compared to nephelometry, smaller studies, cross-sectional studies and those restricted to hip or radiographic OA. This suggests variation between studies is due to both study quality issues and varying methodology. Cross-sectional studies should not be used for causal inference and there were only four longitudinal studies which are stronger methodologically.

Obesity may influence serum hs-CRP levels. Adipose tissue mediates the secretion of pro-inflammatory cytokines, such as IL-6, which in turn triggers the hepatic synthesis of CRP [283, 284, 285]. A previous meta-analysis did not show an association between serum CRP levels and knee OA independent of BMI [277], however, the meta-analysis included only three studies; in contrast, Sower and colleagues reported that difference in CRP levels between participants with knee OA and without knee OA remained statistically significant in both BMI 30 and BMI $>$30 subgroups [270].Our subgroup analysis, which compares studies adjusted for BMI with those did not, suggests that BMI does not explain the variation between studies and the association appears to be independent of BMI. Chronic inflammation, reflected by increased hs-CRP levels, may directly contribute to the occurrence of OA.

With regard to OA phenotypes, our results show a consistent and homogeneous association with pain levels and activity limitations. The magnitude of the association is small suggesting a minor explanatory proportion but would be consistent with a recent paper from our group where CRP predicted the development of knee pain over 5 years [244]. In comparison, the associations between hs-CRP and radiographic OA are not significant.

There are also statistical challenges for data transformation and synthesis. The distribution of hs-CRP values is skewed in most studies. The use of means and standard deviations to calculate the mean difference in the meta-analysis requires an assumption of normal distribution. Because the standard deviation is larger in a skewed distribution, this may weaken

the statistical significance of our results. Furthermore, the majority of studies report crude values of hs-CRP levels while others present the data on the geometric scale. In order to pool data on a common scale, logarithmic back-transformation to the raw scale was required. The method requires small effect sizes and similar distributional shapes in the two groups, which may not be the case in some studies.

Future research is therefore suggested based on this meta-analysis: 1) cohort studies with adequate adjustment for body mass and other common confounding factors such as age, sex and smoking to examine causal relationship between OA structure damage and subsequent elevation of the CRP and other inflammation markers; 2) studies to examine the effects of inflammation alone and in combination with structure abnormalities on OA symptoms; 3) studies using more sensitive OA imaging technologies such as magnetic resonance imaging (MRI) are also desired.

In conclusion, this systematic review suggests that low-grade systemic inflammation may play a greater role in symptoms rather than radiographic changes in OA.

CHAPTER 5

Longitudinal Associations between Adiposity and Change in Knee Pain

5.1 Introduction

Obesity is a well-established risk factor for knee osteoarthritis (OA) and the worldwide obesity epidemic is expected to increase the burden of knee OA [30]. Knee OA commonly presents with knee pain and knee pain is a major musculoskeletal complaint in the elderly [286], therefore, it is important to understand the precise role of obesity in knee pain.

There is limited understanding of the mechanism how obesity is associated with knee pain [287]. Knee pain may be caused by both mechanical-structural factors [287] and metabolic-inflammatory factors [75]. There are a number of cross-sectional studies [161, 288] and longitudinal studies [289, 290] showing that obesity, as defined by increased body mass index (BMI), is associated with knee pain. Unfortunately, the use of BMI alone does not provide adequate information about the metabolic environment that may contribute to the obesity-OA-pain association, because BMI does not discriminate the relative contribution of body fat mass and muscle mass. By using dual energy x-ray absorptiometry (DXA), the amount of fat mass was found to be greater in people with knee pain than in those

without [291]. Assessment of body fat in addition to BMI may help to further elucidate the complex mechanisms in which excess adipose tissue may impact knee pain, though a cross-sectional study suggests that precise measurements of body composition and measures of fat distribution may offer no advantage over the more simple measures of BMI or weight in assessment of risk of radiographic knee OA [292]. There are no longitudinal studies that incorporate body composition assessment using DXA to characterize the association between obesity and knee pain.

We hypothesized that fat mass was associated with increased knee pain over time. Therefore, this study aims to describe the longitudinal association between adiposity assessed in a number of ways and change in knee pain over an average of 5.1 years in older community-living subjects.

5.2 Methods

5.2.1 Study design, setting and participants

This study used the data from the Phase I–III (average 5.1 years) of the Tasmanian Older Adult Cohort (TasOAC) Study as described in Section 3.1.

5.2.2 Knee pain

Knee pain was assessed on the right knee using the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) as described in Section 3.3.

The five pain subscales were grouped into weight-bearing pain (walking on flat surface, going up/down stairs and standing) and non-weight bearing pain (in bed when at night, and sitting/lying) according to the nature of pain [293]. Presence of knee pain was defined as a

pain score of 1 or greater. Change in WOMAC pain score was calculated by subtracting baseline value from follow-up value. Increasing knee pain was defined as a change in pain score of ≥ 1. This outcome definition was based on the calculated minimal clinically important difference (MCID) of 0.9 for WOMAC knee pain for this population [244]. The frequency of knee pain was calculated from the number of time points when knee pain was present. Three levels of frequency were defined as following: 1) consistent knee pain, presence of knee pain at all three time-points (phase 1, 2 and 3); 2) fluctuating knee pain, presence of knee pain in any one or two time-points; 3) no knee pain, WOMAC total pain score = 0 at all time-points.

5.2.3 Anthropometry

Height and weight were measured according to standard protocols as described in Section 3.3. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). Waist circumference measurement (cm) was taken at the level of the mid-point between the inferior margin of the last rib and the crest of the ilium in the mid-auxiliary plane. Hip circumference measurement (cm) was taken at the level of the greatest posterior protuberance of the buttocks and the symphysis pubis.

5.2.4 Body composition

Fat mass was measured using a DXA scanner (Hologic Corp, Waltham, Massachusetts, USA). Percentages of total body fat mass and trunk fat mass were calculated as the ratio of total body or trunk fat mass or lean mass by total body or trunk mass (the sum of fat mass, lean mass and bone mass).

5.2.5 Radiographic OA

Radiographic OA was assessed using the Altman atlas as described in Section 3.5.

5.2.6 Data analysis

Propensity score weighting method was utilized to address missing data in subjects who were lost to follow-up. A weighting score was obtained by the estimation of response propensity based on the baseline characteristics of the participant. The score was used as sample weights in subsequent statistical analyses. Partial correlation analyses were performed to measure the relationship between BMI and body composition after adjustment for age and sex. Standardization of the coefficients was carried out in order to compare the effects between different measures of obesity.

Statistical analyses were performed to evaluate the association of baseline BMI, waist circumference, waist/hip ratio, body fat mass and lean mass with frequency of knee pain and increase in WOMAC pain scores over follow-up period. Log multinomial regression was used to analyze the frequency of knee pain (consistent or fluctuating knee pain vs no knee pain) and log-binomial regression was used to analyze the increase in WOMAC pain scores (increase vs no increase). Multivariable analyses were adjusted for age, sex, height (not for BMI) and radiographic OA. Sensitivity analyses were performed to examine if the results were the same using different cut-offs for the increase in knee pain ($\geq$ 2, 3, 4 and 5).

To fully utilize the longitudinal data of multiple obesity measures and knee pain scores, a linear mixed-effect model with subjects as a random effect was employed to analyze the association between change in obesity indicators and change in WOMAC total pain score. The number of years between the baseline and the follow-up visit was used as a time variable.

5.3 Results

5.3.1 Descriptive analyses

A total of 767 participants (69.8%) had complete knee pain data. 49 of them did not have knee X-ray at baseline. Compared to those lost to follow-up, study participants were younger, less obese as assessed by BMI, had less body fat mass and more lean mass, and had lower WOMAC total pain scores at baseline. The prevalence of knee pain was comparable between the two groups (Table 5.1).

Table 5.1: Baseline characteristics by those who did and did not complete the follow-up.

Baseline Characteristics	Complete follow-up (n = 767)	Loss to follow-up (n = 332)	P value
Age (years)	61.6 (7.00)	64.7 (8.14)	<0.001
Female	381 (50%)	181 (55%)	0.140
BMI (kg/m^2)	27.7 (4.58)	28.4 (5.11)	0.027
<25 kg/m^2	229 (30%)	89 (27%)	0.307
25 – 29.9 kg/m^2	340 (44%)	138 (42%)	0.397
>30 kg/m^2	198 (26%)	105 (32%)	0.048
Weight (kg)	77.8 (14.60)	78.1 (15.85)	0.733
Waist circumference (cm)	93.4 (12.76)	95.8 (13.77)	0.004
Body fat (%)	33.5 (7.94)	35.3 (8.25)	0.001
Lean mass (%)	63.5 (7.64)	61.8 (7.92)	0.001
Knee Pain	392 (51%)	190 (58%)	0.051
Total WOMAC pain	3.3 (5.69)	4.9 (7.74)	<0.001
Radiographic OA*	423 (59%)	187 (61%)	0.654
Joint space narrowing*	418 (59%)	187 (61%)	0.511
Osteophyte*	69 (10%)	30 (10%)	0.968

WOMAC, Western Ontario and McMaster University Osteoarthritis Index;
Results are shown as mean (standard deviation) or number of participants (percentage) unless stated otherwise;
Bold p values indicate statistically significant difference between study participants and loss to follow-up at α=0.05;
**49 participants in the complete follow-up and 18 participants in the loss to follow-up did not have an x-ray at baseline.*

The partial correlations are 0.48 ($p<0.01$) and 0.65 ($p<0.01$) for BMI with total body fat percentage and trunk fat percentage, respectively. Over the average follow-up period of 5.1

years, the mean change in WOMAC total pain score was -0.5 (standard deviation 5.1, range -36 to 24). 175 subjects (23%) had increased pain score from baseline to follow-up. Table 5.2 presents the comparison of baseline characteristics between study participants with and without any increase in knee pain score. Compared to those without increased knee pain, participants with increased knee pain score had higher BMI, weight, waist circumference, body fat percentage and trunk fat mass. There were no significant differences in age, sex and baseline pain score between the two groups.

Table 5.2: Baseline characteristics between participants with and without increased pain over an average of 5.1 years (n = 766)

Baseline Characteristics	Increase in pain (n = 175)	No increase in pain (n = 591)	P value
Age (year)	62.4 (7.16)	61.9 (6.97)	0.46
Female	95 (54%)	285 (48%)	0.16
BMI (kg/m^2)	29.1 (5.25)	27.3 (4.27)	<0.01
Total WOMAC pain	2.96 (4.9)	3.35 (5.9)	0.42
Radiographic OA*	111 (68%)	312 (57%)	0.01
Joint space narrowing*	109 (67%)	309 (56%)	0.02
Osteophyte*	33 (22%)	33 (6%)	<0.01
Anthropometry			
Weight (kg)	80.8 (14.17)	76.9 (14.63)	<0.01
WC (cm)	96.0 (12.53)	92.6 (12.73)	<0.01
W/H ratio	0.92 (0.08)	0.92 (0.09)	0.49
Body composition			
Body fat (kg)	30.2 (7.83)	27.0 (7.83)	<0.01
Trunk fat (kg)	13.7 (4.84)	12.1 (4.31)	<0.01
Lean mass (kg)	52.9 (9.65)	52.4 (10.65)	0.62

Results are shown as mean (standard deviation) or the number of participants (percentage) unless stated otherwise.
BMI, body mass index; WC, waist circumference; W/H ratio, waist-to-hip ratio;
Bold p values indicate statistically significant difference at α=0.05.
**12 participants with an increase in pain and 37 participants with no increase in pain did not have an x-ray at baseline.*

Table 5.3: Baseline obesity measures and increase in knee pain over 5.1 years.

	Univariable analysis Relative risk (95% CI) (n = 767)		Multivariable analysis§ Relative risk (95% CI) (n = 718)	
BMI (per SD)	1.33	(1.19, 1.48)*	1.34	(1.20, 1.49)*
Weight (per SD)	1.21	(1.08, 1.36)*	1.41	(1.22, 1.64)*
WC (per SD)	1.24	(1.09, 1.41)*	1.37	(1.18, 1.59)*
W/H ratio (per SD)	1.04	(0.92, 1.18)	1.23	(1.03, 1.47)*
Total fat (per SD)	1.34	(1.20, 1.51)*	1.36	(1.20, 1.55)*
Trunk fat (per SD)	1.31	(1.16, 1.49)*	1.32	(1.15, 1.50)*

WOMAC, Western Ontario and McMaster University Osteoarthritis Index;
BMI, body mass index; WC, waist circumference; W/H ratio, waist/hip ratio; SD, standard deviation;
§All multivariable analyses are adjusted for age, gender, height (not for BMI) and radiographic OA;
** P value <0.05*

5.3.2 Baseline obesity measures and increasing knee pain

The relative risk of increase in knee pain (developing knee pain and having worsening knee pain) associated with adiposity is presented in Table 5.3. There were no significant interactions between sex and obesity measures on knee pain (data not shown), therefore data from females and males were combined for analyses. Except for waist-to-hip ratio, all baseline obesity indicators were significantly associated with increasing knee pain in both univariable and multivariable analyses with the association for total fat mass being marginally stronger than the other obesity indicators. Lean mass was associated with increase in pain, however, the association became non-significant after adjustment for fat mass (data not shown). The results were very similar if different cut-offs were used to defined an increase in knee pain.

Figure 5.1 and 5.2 show the association between different adiposity measures and increasing knee pain in each WOMAC subscale after adjustment for covariates. The relative risks for weight-bearing pains are present in Figure 5.1 and the relative risks for non-weight-bearing pains are present in Figure 5.2. BMI was the strongest and most consistent predictor of increased pain scores in both weight-bearing and non-weight-bearing pain. The associations between body fat mass, trunk fat mass and increasing weight-bearing knee pain subscales

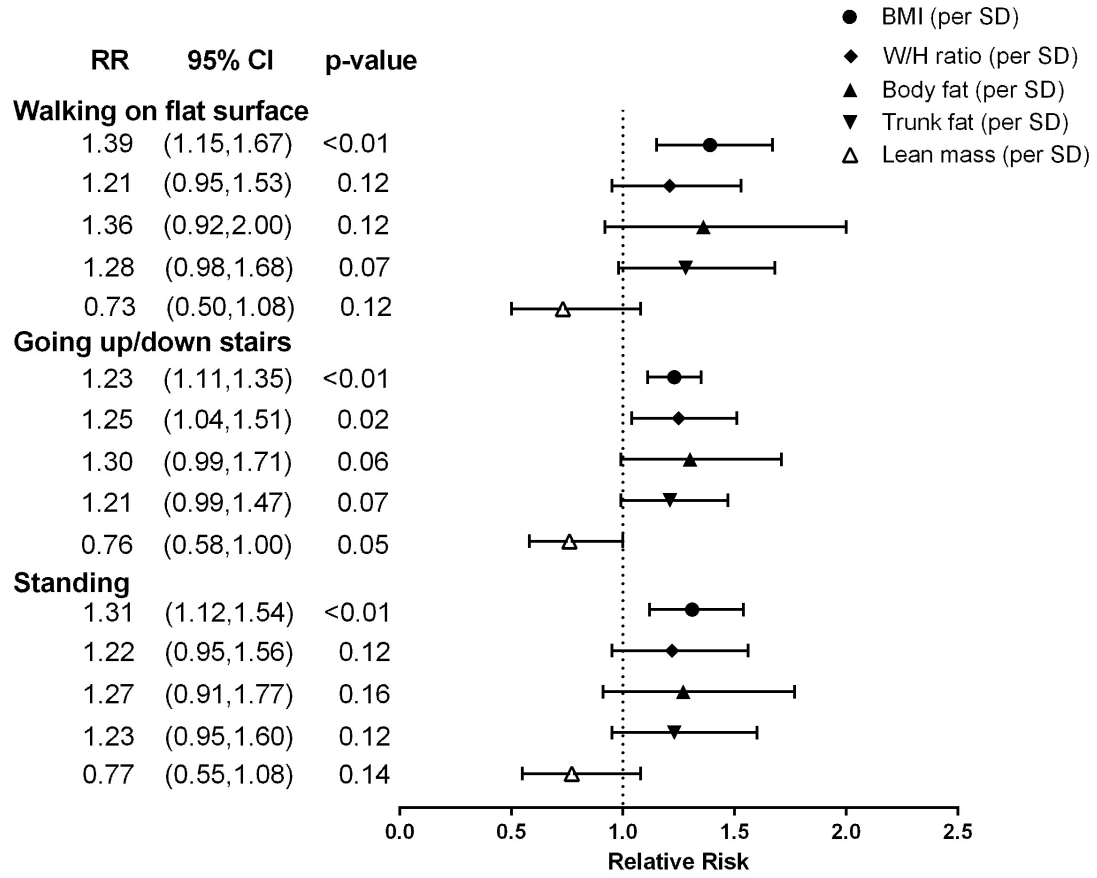
were not statistically significant (Figure 5.1), but surprisingly, was for non-weight-bearing knee pain subscales (Figure 5.2). Waist-to-hip ratio was significantly associated with weight-bearing knee pain going up/down stairs and non-weight-bearing knee pain sitting.

5.3.3 Baseline obesity measures and consistency of knee pain

Figure 5.3 describes the association between obesity measures at baseline and consistency of knee pain in a multinomial logistic regression model. Participants who were pain-free for the entire course of the study were used as the reference group. Higher BMI, heavier weight, larger waist circumference and more fat mass were associated with both consistent and fluctuating knee pain over follow-up. A larger waist-to-hip ratio was significantly associated with fluctuating knee pain but not with consistent knee pain. Among all adiposity measures, total fat mass percentage was the strongest predictor for both consistent and fluctuating knee pain.

5.3.4 Mixed modeling between obesity measures and knee pain

The results of the analyses are presented in Table 5.4. In order to compare the strength of associations across different obesity measures, standardized coefficients were calculated. All fat measures were significantly and positively associated with WOMAC total pain score. After adjustment for common covariates, one standard deviation increase in BMI was associated with 1.27 ($p<0.01$) unit increase in WOMAC total pain score. Similarly, one standard deviation increase in total body fat resulted in 1.17 ($p<0.01$) higher in WOMAC total pain score. The results remained largely unchanged when radiographic OA was not included in the statistical models as an adjusting covariate (data not shown).



BMI, body mass index; W/H ratio, waist/hip ratio; SD, standard deviation.

Figure 5.1: Adiposity and weight-bearing pain.

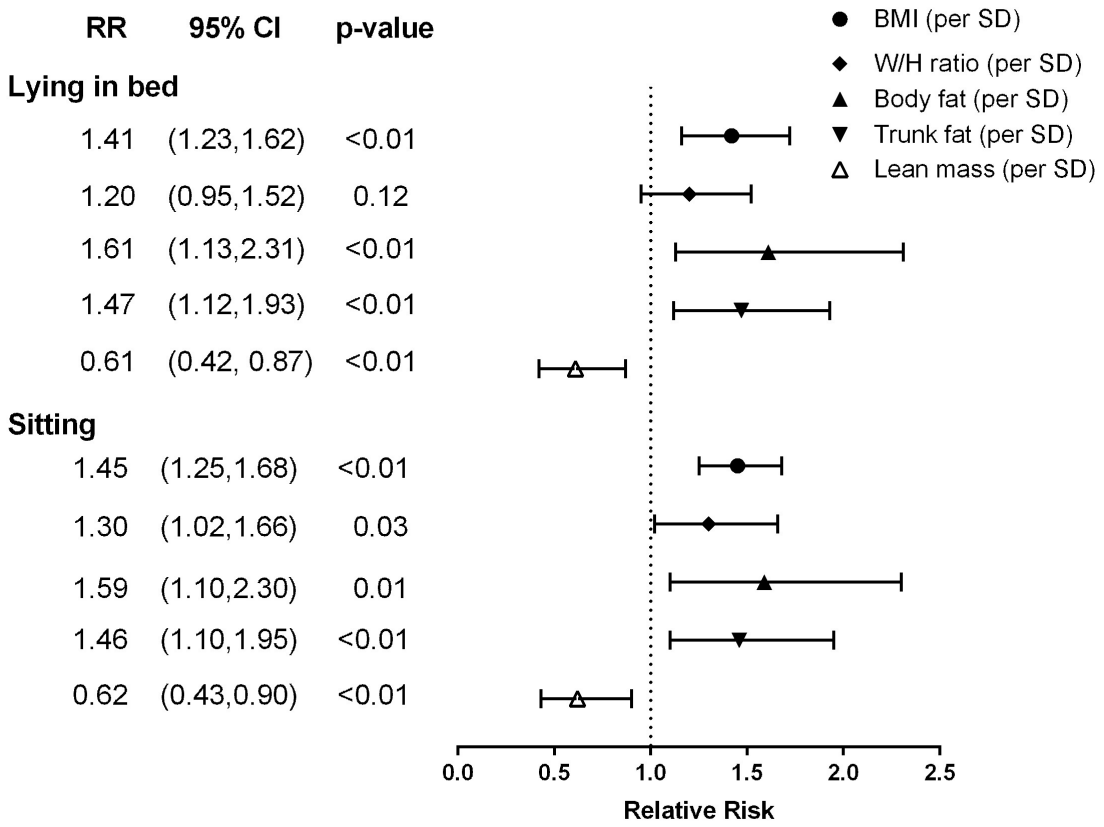
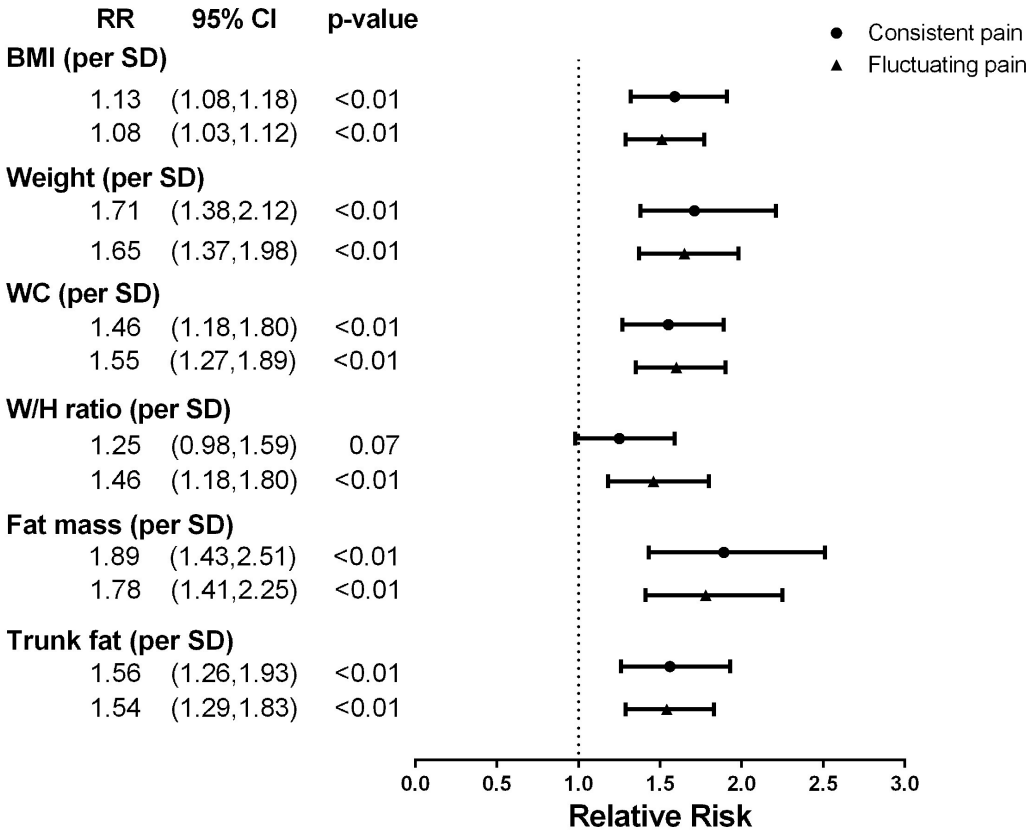


Figure 5.2: Adiposity and non-weight-bearing pain.



BMI, body mass index; WC, waist circumference; W/H ratio, waist/hip ratio; SD, standard deviation

Figure 5.3: Obesity measures, body composition and frequency of knee pain over 5.1 years (compared to no pain).

Table 5.4: Mixed modeling of the association between obesity measures and repeated total WOMAC pain measures over 5.1 years.

	Univariable analysis Standardized β (95% CI) (n = 767)		Multivariable analysis§ Standardized β (95% CI) (n = 718)	
BMI (per SD)	1.15	(0.87, 1.44)*	1.27	(0.89, 1.66)*
Weight (per SD)	0.74	(0.45, 1.04)*	1.46	(1.02, 1.90)*
WC (per SD)	0.82	(0.54, 1.09)*	1.38	(0.97, 1.80)*
W/H ratio (per SD)	0.34	(0.08, 0.62)*	1.36	(0.83, 1.90)*
Total fat (per SD)	0.92	(0.65, 1.20)*	1.17	(0.76, 1.59)*
Trunk fat (per SD)	0.84	(0.57, 1.12)*	1.00	(0.61, 1.39)*

WOMAC, Western Ontario and McMaster University Osteoarthritis Index;
BMI, body mass index; WC, waist circumference; W/H ratio, waist/hip ratio; SD, standard deviation;
§All multivariable analyses are adjusted for age, gender, height (not for BMI) and radiographic OA;
** P value <0.05*

5.4 Discussion

Our study adds to previous cross-sectional studies by examining the longitudinal association between adiposity measures and change in knee pain over an average of 5.1 years. Our results suggest that adiposity measures at baseline are significant predictors of consistent and increasing knee pain over time. In addition, the change in adiposity measures corresponds to the change in WOMAC total pain score in time series analyses. Obesity measures, including BMI, waist circumference, waist-to-hip ratio, and percentage body fat, are deleteriously associated with knee pain in older adults. This study also suggests that body fat mass is more consistently associated with non-weight-bearing than weight-bearing knee pain, suggesting metabolic and inflammatory mechanisms may underlie knee pain.

Obesity-specific mechanisms associated with knee pain include mechanical stress, systemic inflammation and relative loss of muscle mass and strength [294]. As reported in a systematic review [19], being overweight and obese is associated with incident OA and this may be a result of increased loading, which affects the knee joint structure and biomechanical properties of the joint. Apart from mechanical causes, increased adipose tissues may exert a pro-

inflammatory and metabolically active effect, by producing cytokines and adipokines, such as interleukin (IL)-6, tumor necrosis factor (TNF)-α, and leptin [69]. Our previous studies have shown that the serum levels of these cytokines and adipokines are independently associated with cartilage loss and may play an important role in the diseases process of OA [244, 295, 296]. Adiposity might also have a role in the perception of pain. Pro-inflammatory cytokines IL-6 and TNF-α secreted by adipose tissue can act as pain modulators to induce production of histamine and substance P [297], which contribute to the more musculoskeletal complaints seen in obese people [298]. Epidemiological data have shown that the levels of TNF-α, IL-6 and high sensitivity C-reactive protein (CRP) are associated with increased knee pain [244]. Although previous research has not examined adipose tissue in relation to knee pain, a number of studies have reported that increased fat mass and central obesity are associated with the severity of pain from other body regions [299, 300]. The results of the current study support the hypothesis that obesity, reflected by increased BMI and body fat mass, is associated with knee pain through multiple mechanisms. BMI, as an obesity measure, may reflect both metabolic effects of adiposity and increased loading on the knee joint. Indeed, BMI was highly correlated with total body fat and trunk fat, and was consistently associated with total knee pain, weight-bearing and non-weight-bearing knee pain, suggesting both mechanic and metabolic mechanisms are involved in the pathogenesis. Compared to BMI, the results for body fat mass percentage and non-weight-bearing pain suggest a potential systemic effect.

Our results showed that one standard deviation (4.6 kg/m^2) increase in BMI at baseline would result in 34% more risk or one standard deviation (8.7 kg) increase in total body fat mass at baseline would result in 36% more risk of having an increase in knee pain over average 5.1 years. Although these differences over 1 year would be modest, the clinical significance is high at a population level given the high prevalence of overweight. Although males and females are known to differ their adiposity deposition, we did not find significant

interactions between adiposity measures and gender. This suggests that the effects of adiposity on pain could be the same in both genders. In general, females have more fat mass than males and adiposity may account for the higher prevalence of knee pain in women [30].

The major limitation of our study is potential selection bias resulting from loss to follow-up. The retention rate after follow-up was 69.7%. Those who remained in the study were younger (61.6 versus 64.7 years) and less obese (26% versus 32%) than those lost to follow-up. However, sensitivity analyses using inverse propensity weighting did not significantly differ from the results of the original analyses. Thus our results are generalizable to this population. In addition, such bias may have underestimated our findings given that age and obesity are associated with increased risk of knee pain.

Although the present study has identified significant associations between adiposity and knee pain, the exact underlying mechanisms for the relationship between adiposity and knee pain are beyond the scope of this study. More studies are needed to explore the causal pathways between obesity and knee pain in the future.

In conclusion, BMI is the most consistent correlate of knee pain in older adults suggesting simple measures may provide the most information. Fat mass is only associated with non-weight-bearing knee pain suggesting mechanisms other than mechanical load are important.

CHAPTER 6

Effect of Vitamin D Supplementation on Symptomatic Knee OA: a Randomized Clinical Trial

6.1 Introduction

Symptomatic knee osteoarthritis (OA) occurs in 10% men and 13% women aged 60 years or older [301]. Worldwide, knee OA together with hip OA are the 11th leading cause of global disability, accounting for 2.2% of total years lived with disability [2]. Medical costs of OA account for 1% to 2.5% of the gross domestic product in developed countries [302]. Currently, there are no disease-modifying therapies for knee OA; therefore, there is an urgent need to develop cost-effective approaches to prevent the development and progression of this disease.

Vitamin D can reduce bone turnover and cartilage degradation, thus potentially preventing the development and progression of knee OA [122, 129]. Epidemiological studies showed that low serum 25-hydroxyvitamin D (25OHD) levels were associated with greater knee pain [127, 128], a higher prevalence of radiographic knee OA [120], as well as higher risk of progression [124, 125]. While observational data is promising, these studies are subject to in-

herent bias and confounding factors such as physical activity and sun exposure [303]. In addition, two small existing randomized controlled trials (RCTs) reported contradictory results [129, 130]. The inconsistencies are likely due to variations in inclusion criteria, outcome measures, follow-up time and sample size. An updated systematic review called for further larger well-designed RCTs to determine whether vitamin D supplementation can slow disease progression [304]. Therefore, we designed an RCT, addressing the shortcomings of the previous studies by recruiting a sufficient sample size of participants with clinically relevant inclusion criteria [305], to evaluate the effects of vitamin D supplementation over two years versus placebo on knee pain and knee cartilage volume in symptomatic knee OA patients with low 25OHD levels. Effects on knee physical function and other knee structural abnormalities, including cartilage defects and bone marrow lesions, were also assessed.

6.2 Method

6.2.1 Trial design, setting and participants

This study used the primary data from the Vitamin D Effect on Osteoarthritis (VIDEO) study. The details of the study are described in Section 3.2

6.2.2 Outcomes

Primary outcome measures were change in knee pain assessed using the Western Ontario and McMaster University Index of OA (WOMAC) score [230] and change in tibial cartilage volume on MRI from baseline to month 24 [306]. Secondary outcomes included cartilage defects and BMLs on MRI. Post hoc analysis outcomes include 20% and 50% improvement rates in WOMAC pain score, WOMAC function, visual analogue scale knee pain, and the responder criteria developed by the Outcome Measures in Rheumatology Arthritis Clinical

Trials-Osteoarthritis Research Society International (OMERACT-OARSI) criteria.

6.2.3 Assessment of pain

Knee pain was assessed at baseline and at month 3, 6, 12 and 24. Five items of WOMAC pain scale in 100 mm visual analogue format were used to assess pain during walking, using stairs, in bed, sitting or lying, and standing. Items were summed to create a total pain score (range 0-500) [307]. Knee pain in most days of last month was assessed using a 100 mm visual analogue scale.

6.2.4 WOMAC

Total WOMAC score is the sum of subscale scores including pain, stiffness and physical function. Missing responses were managed according to the WOMAC user guide.[21] The WOMAC pain score was considered invalid if more than one item was missing. In the event of a missing item, the remaining four items were averaged and then multiplied by five.

6.2.5 OMERACT-OARSI Responder Criteria

The OARSI Standing Committee for Clinical Trials Response Criteria Initiative developed a set of responder criteria (OMERACT-OARSI) to categorize individual response to treatment as a single variable for clinical trials [308]. Response using the exact OMERACT-OARSI criteria could not be directly evaluated because the patient global assessment was not recorded in this trial, therefore, we employed a modified OMERACT-OARSI responder definition without patient global assessment. OMERACT-OARSI responders in our study were defined as participants with (i) $\geq$ 50% improvement and an absolute change of at least 20 points in the mean WOMAC pain score or mean WOMAC function score, or (ii) $\geq$ 20%

improvement and an absolute change of at least 10 points in both the mean WOMAC pain score and the mean WOMAC function score.

6.2.6 MRI assessment of knee structural changes

MRI scans of the study knee were obtained according to a standardized protocol as described in Section 3.6

Cartilage volume

Cartilage volume was determined using the previously described image processing techniques [120]. The volumes of individual cartilage plates (medial tibial and lateral tibial) were isolated by manually drawing disarticulation contours around the cartilage boundaries on a section-by-section basis then resampled by means of bilinear and cubic interpolation for final 3D rendering using OsiriX Lite imaging software (32-bit version 5.9, Pixmeo SARL, Geneva, Switzerland). The coefficient of variation (CV) was 2.1% for medial tibia and 2.2% for the lateral tibia [309].

Cartilage defects

Cartilage defects (0–4) were graded on T2-weighted images using a modified Outerbridge classification [306] at medial tibial, medial femoral, lateral tibial and lateral femoral sites: grade 0, normal cartilage; grade 1, focal blistering and intracartilaginous hyperintensity with an normal contour; grade 2, irregularities on the surface and loss of thickness of less than 50%; grade 3, deep ulceration with loss of thickness of more than 50% without exposure of subchondral bone; grade 4, full-thickness chondral wear with exposure of subchondral bone. A total score was calculated as the total of subregional scores. Intra-observer reliability

expressed as the intra-class correlation coefficient (ICC) ranged from 0.77 to 0.94.

Bone marrow lesions

Bone marrow lesions were defined as discrete areas of increased signal adjacent to the subcortical bone and measured using modified Whole-Organ Magnetic Resonance Imaging Score (WORMS)25: 0=none; 1 $\leq$ 25% of the subregion; 2 = 25–50%; 3 $\geq$ 50%. A total score of the tibiofemoral compartment was calculated as the total of 13 subregional scores (0–39). The ICC of this bone marrow lesion ranged from 0.93 to 0.98.

6.2.7 25OHD assays

Serum 25OHD was assayed at screening, month 3 and 24, utilizing a direct competitive chemiluminescent immunoassay (DiaSorin Inc., Stillwater, Minnesota, USA). The intra-assay and inter-assay coefficients of variation were 3.2% and 6.0%.

6.2.8 Sample size

The sample size calculation is described in Section 3.2.6

6.2.9 Statistical analysis

WOMAC and VAS knee pain scores were analyzed using a repeated measures mixed model with terms for age, sex, body mass index, treatment, month and trial center. The correlation within the repeated measures was addressed by using individual participant ID as a random effect. The effect of treatment was evaluated by the month $\times$ treatment interaction. In a post-hoc analysis, we evaluated the proportion of participants who achieved at least 20% and 50% improvement in WOMAC pain score, which has been shown to be clinically relevant [310].

The independent t-test was used to compare annual changes in cartilage volume and absolute changes of cartilage defects and bone marrow lesions between groups. An increase in cartilage defects and bone marrow lesions was defined as a change of more than one unit in the score. The presence of an increase in cartilage defects or bone marrow lesions was compared between the two groups using logistic regression.

Both intention to treat and per protocol analyses were utilized. Per protocol was defined as achieving a 25OHD level >60 nmol/L at the month 3 visit. Multiple imputations by chained equations was used to address missing data due to loss to follow-up and non-responses. Imputations were performed separately for each treatment group and each outcome, using baseline values, age, gender, body mass index and serum 25OHD level.

6.3 Results

6.3.1 Participants

Figure 3.2 in the Section 3.2.1 shows the flow of the study. A total of 599 participants were screened for eligibility from 5 Jun 2010 to 1 Dec 2011 and 413 participants were randomly assigned to either vitamin D or placebo. The mean age of participants was 63.2 years, with 208 (50%) females and a mean body mass index of 29.6 kg/m^2. Participants demographic characteristics were comparable at baseline between two groups (Table 6.1). Twenty-eight participants (13.4%) in the vitamin D group and 45 (22.1%) in the placebo group withdrew from the study (p = 0.021). 340 participants (82.3%) completed the trial. There were no significant differences between those who completed and those who withdrew apart from more females and lower tibial cartilage volume in those who withdrew (Table 6.2). Fewer participants discontinued treatment in the vitamin D group than the placebo group (8 versus 21). The major reason for a higher drop-out rate in the placebo group was that participants

had their 25OHD levels checked by a primary health provider and started taking vitamin D after finding low vitamin D levels. Consequently, they were withdrawn from the study. All available data from the randomized participants were analyzed in the intention-to-treat analyses.

Table 6.1: Baseline characteristics of the vitamin D and the placebo groups.

	Vitamin D (n=209)	Placebo (n=204)
Hobart	129 (61%)	132 (64%)
Melbourne	80 (38%)	72 (35%)
Age (years)	63.5 (6.9)	62.9 (7.2)
Female (%)	106 (50%)	102 (50%)
BMI (kg/m^2)	29.6 (5.4)	29.6 (4.6)
Serum 25OHD (nmol/L)	43.7 (11.8)	43.8 (12.7)
Radiographic OA (%)	163 (96%)	157 (96%)
Total WOMAC score (0–2400)	687.3 (426.3)	664.7 (390.8)
Pain (0–500)	137.8 (88.9)	134.4 (83.2)
Stiffness (0–200)	61.5 (41.6)	61.7 (40.1)
Function (0–1700)	487.9 (318.1)	467.6 (292.8)
VAS pain (0–100)	51.4 (18.8)	49.6 (17.8)
Tibial cartilage volume (cm^3)	3.47 (1.04)	3.64 (1.04)
Medial tibial region (cm^3)	1.46 (0.46)	1.52 (0.47)
Lateral tibial region (cm^3)	2.01 (0.72)	2.12 (0.68)
Total bone area (cm^2)	32.8 (5.4)	32.6 (5.1)
Cartilage defects (%)	194 (93%)	192 (94%)
Medial tibiofemoral region (%)	176 (84%)	164 (80%)
Lateral tibiofemoral region (%)	159 (76%)	157 (77%)
Bone marrow lesions (%)	134 (64%)	147 (72%)
Medial tibiofemoral region (%)	90 (43%)	95 (46%)
Lateral tibiofemoral region (%)	81 (39%)	89 (43%)

BMI, body mass index; 25OHD, 25-hydroxyvitamin D; OA, osteoarthritis;
WOMAC, Western Ontario and McMaster University Index; VAS, visual analogue scale;
Higher scores in WOMAC and VAS pain indicate a more severe stage of the condition.
Results are shown as mean (SD) or number (percentage) unless stated otherwise.

Table 6.2: Baseline characteristics of those completed and dropouts.

	Completed (n=340)	Dropout (n=73)	P-value
Hobart	214 (62.9%)	47 (64.4%)	0.817
Melbourne	126 (37.1%)	26 (35.6%)	
Vitamin D	181 (53.2%)	28 (38.4%)	0.021*
Placebo	159 (46.8%)	45 (61.6%)	
Age (years)	63.2 (7.1)	63.1 (7.0)	0.886
Female (%)	158 (46.5%)	50 (68.5%)	<0.001*
BMI (kg/m^2)	29.5 (4.9)	30.0 (5.7)	0.408
Serum 25OHD (nmol/L)	44.0 (12.1)	42.5 (13.0)	0.334
Radiographic OA (%)	279 (95.6%)	60 (98.4%)	0.306
Total WOMAC score (0-2400)	662.4 (405.7)	741.7 (420.6)	0.138
Pain (0–500)	133.8 (86)	148.2 (86)	0.197
Stiffness (0–200)	61.3 (40.4)	63.2 (42.9)	0.710
Function (0–1700)	467.2 (302.8)	529.1 (316.5)	0.121
VAS pain (0–100)	47.1 (20.7)	48.6 (22.7)	0.593
Tibial cartilage volume (cm^3)	3.63 (1.05)	3.20 (0.93)	0.002*
Cartilage defects (%)	330 (97.6%)	69 (97.2%)	0.824
Bone marrow lesions (%)	267 (79.0%)	58 (81.7%)	0.609

BMI, body mass index; 25OHD, 25-hydroxyvitamin D; OA, osteoarthritis;
WOMAC, Western Ontario and McMaster University Index; VAS, visual analogue scale;
Results are shown as mean (SD) or number (percentage) unless stated otherwise.
Student t-test or Chi^2 test was used for the comparison.
Asterisk signs indicate statistical significance.

6.3.2 Primary endpoints

25OHD level increased more in the vitamin D group (40.6 vs. 6.7 nmol/L, p <0.001) over two years. Overall, 165 (79%) participants in the vitamin D group and 88 (43%) participants in the placebo group reached a 25OHD level over 60 nmol/L at month 3.

Changes in WOMAC knee pain are presented in Table 6.3. At baseline, the mean and standard deviation of WOMAC pain score were 137.9 ± 88.8 in the vitamin D group and 134.7 ± 83.4 in the placebo group (95% CI of difference [-13.5, 19.8], p = 0.712). Total WOMAC pain decreased over 24 months in both groups (Figure 6.1). At month 24, the mean and standard deviation of WOMAC pain score were 87.0 ± 90.1 in the vitamin D group and 97.2 ±

87.5 in the placebo group (95% CI of difference [-28.8, 8.4], p = 0.283). There was no difference in change in WOMAC pain between groups in the mixed-effect model in which all time-points were included (-49.9 vs. -35.1, between-group difference 14.8, 95% CI [-32.5, 2.9], p = 0.102).

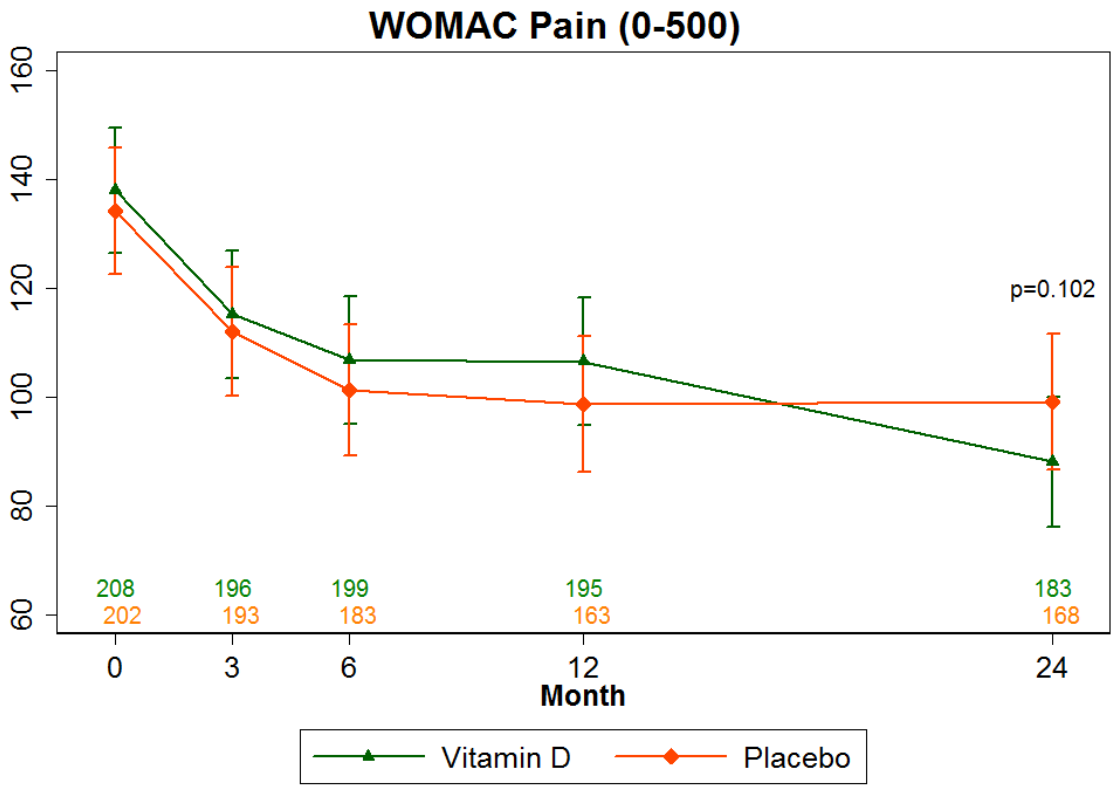


Figure 6.1: Comparison between vitamin D and placebo on change in WOMAC pain.

Tibial cartilage volume (mean ± standard deviation) was not different between the vitamin D group (3466 ± 1038 mm^3) and the placebo group (3640 ± 1036 mm^3) at baseline (between-group difference -174 mm^3, 95% CI of difference [-375, 27], p = 0.091). At month 24, tibial cartilage volume was also not different between the vitamin D group (3238 ± 989 mm^3) and the placebo group (3398 ± 1030 mm^3) (between-group difference -160 mm^3, 95% CI of difference [-369, 49], p = 0.134). Change in tibial cartilage volume (Table 6.3) was not different between the groups (-242.6 mm^3 vs. -301.4 mm^3, between-group difference 58.8

mm^3, 95% CI [-13.9, 131.4], p = 0.114). Per protocol analysis comparing those achieved 25OHD level >60 nmol/L at month 3 visit to those who did not (253 vs. 146, 14 participants withdrew within 3 months) showed similar results (-261.9 mm^3 vs. -284.8 mm^3, between-group difference 22.9 mm^3, 95% CI [-51.5, 97.3], p = 0.547).

6.3.3 Secondary endpoints

The results for tibiofemoral cartilage defects and bone marrow lesions are shown in Table 6.3. The difference in cartilage defect score was not statistically significant between groups. Bone marrow lesion scores decreased in both groups and no significant difference was observed.

6.3.4 Post-hoc analyses

In post-hoc analyses (Table 6.3), participants in the vitamin D group had statistically significant improvements in VAS knee pain (Figure 6.2) scores compared to the placebo group. The vitamin D group had more improvement in total WOMAC score (Figure 6.3) and WOMAC function (Figure 6.4), but not WOMAC stiffness (Figure 6.5). There were 115 (64%) participants in the vitamin D group and 95 (57%) participants in the placebo group (p = 0.164) who achieved a 20% improvement in WOMAC knee pain score over two years. There were 90 (50%) participants in the vitamin D group and 65 (39%) participants in the placebo group (p = 0.036) that showed at least a 50% improvement in WOMAC pain. Furthermore, there were significantly more OMERACT-OARSI responders in the vitamin D group than the place group (35% versus 25%, p = 0.029). However, the proportion of participants that had an increase in bone marrow lesions was significantly lower in vitamin D group (17% versus 27%, p = 0.031).

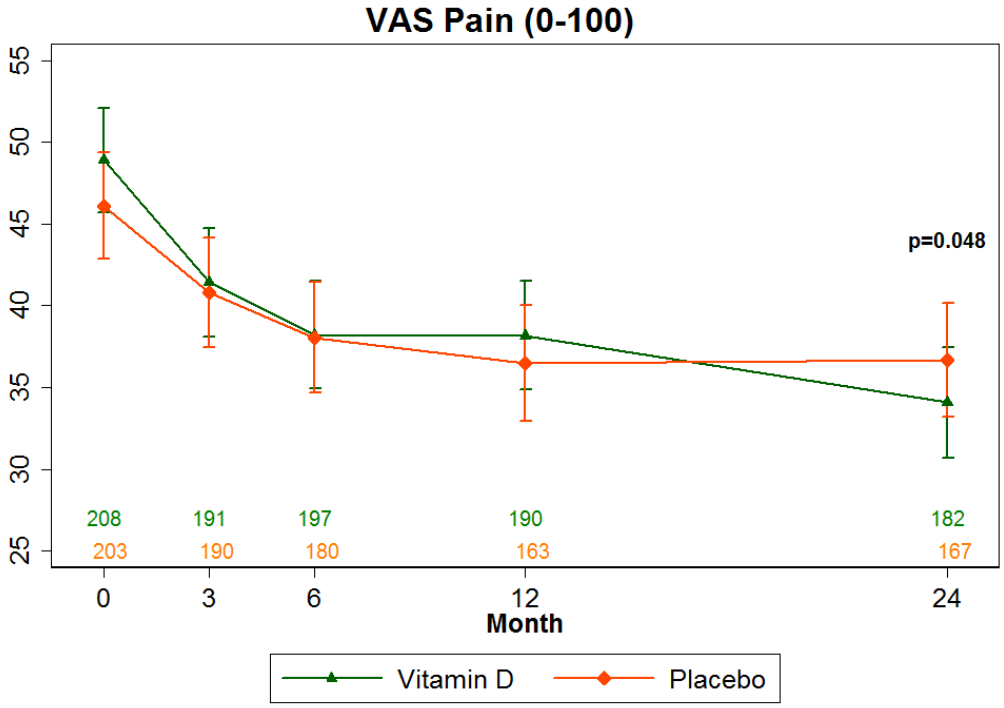


Figure 6.2: Comparison between vitamin D and placebo on change in VAS pain.

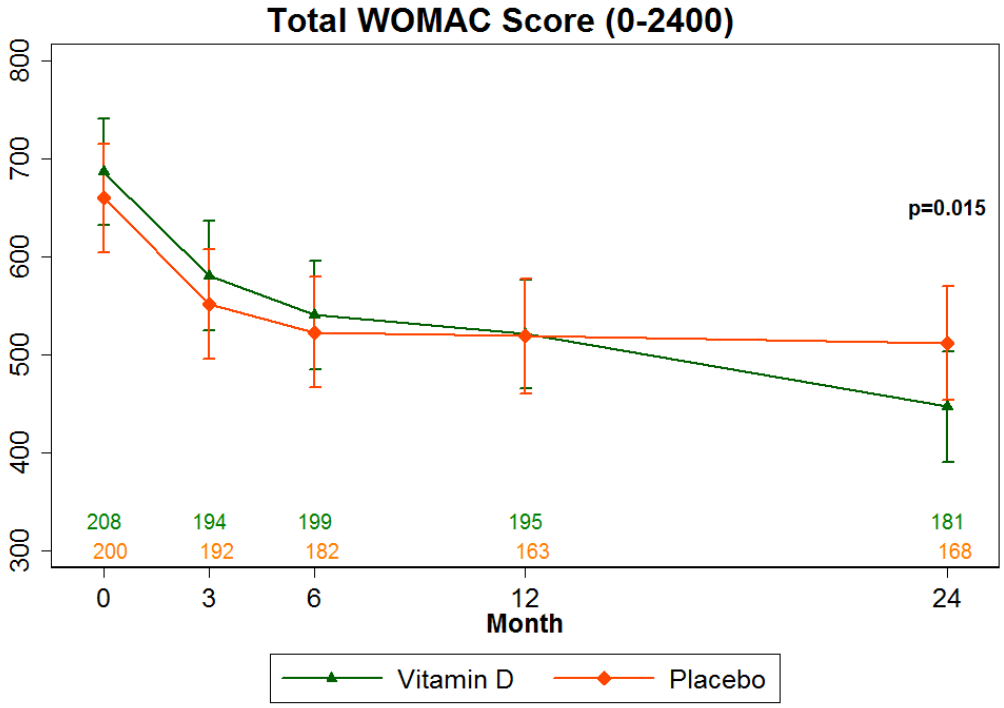


Figure 6.3: Comparison between vitamin D and placebo on change in total WOMAC score.

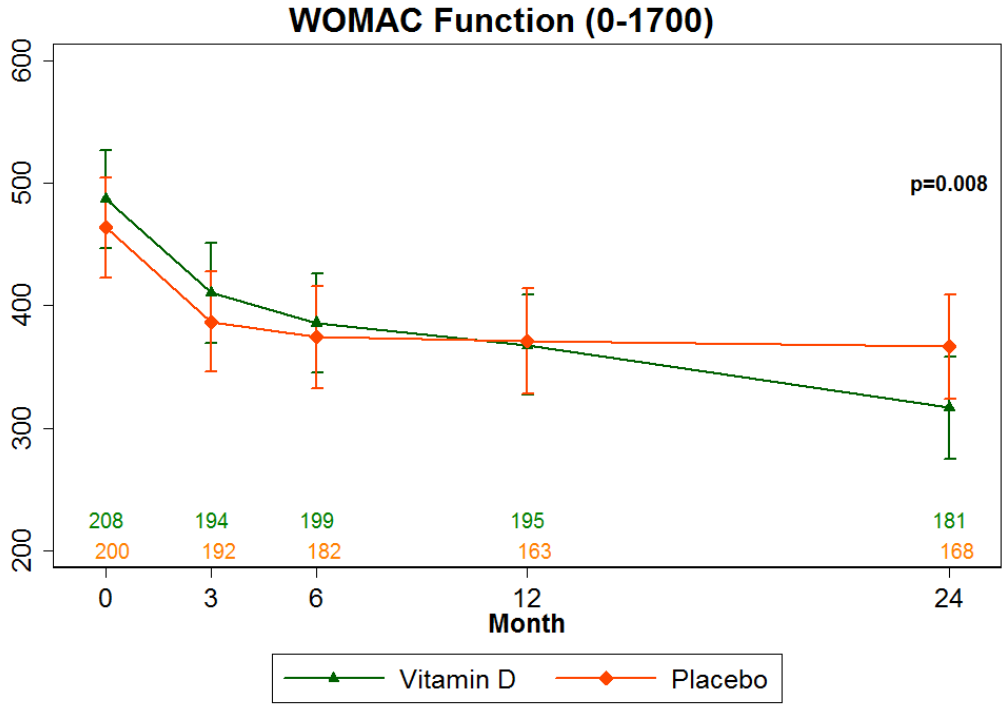


Figure 6.4: Comparison between vitamin D and placebo on change in WOMAC function.

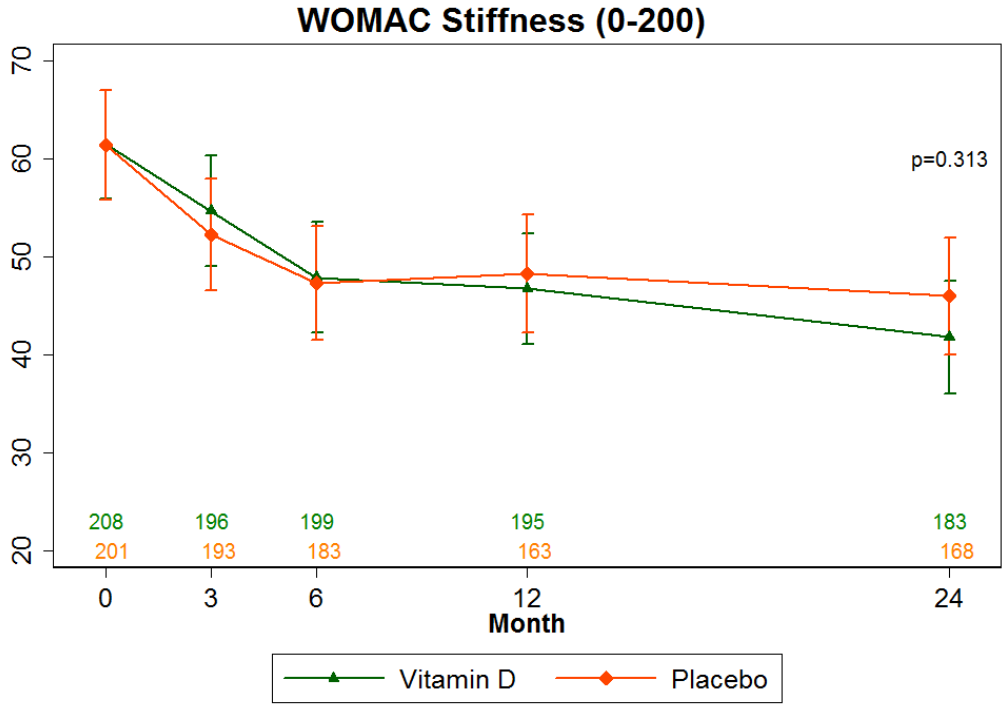


Figure 6.5: Comparison between vitamin D and placebo on change in WOMAC stiffness.

Table 6.3: Change in study endpoints over two years between vitamin D and placebo [mean (95% CI)]

	Vitamin D (n = 209)		Placebo (n = 204)		Between-group difference in change†‡	P-value
	Baseline	Month 24	Baseline	Month 24		
Primary Endpoints						
WOMAC pain (0–500)	137.9 ± 88.8	87.0 ± 90.1	134.7 ± 83.4	97.2 ± 87.5	-14.8 (-32.5, 2.9)	0.102
Tibial cartilage volume (mm^3)	3466 ± 1038	3238 ± 989	3640 ± 1036	3398 ± 1030	58.78 (-13.86, 131.40)	0.114
Medial tibial cartilage volume (mm^3)	1461 ± 463	1369 ± 464	1522 ± 474	1407 ± 456	31.68 (-10.04, 73.38)	0.138
Lateral tibial cartilage volume (mm^3)	2005 ± 716	1870 ± 673	2118 ± 681	1992 ± 689	26.70 (-19.44, 72.86)	0.258
Change in tibial cartilage(% p.a.)	-3.44 (-4.17, -2.72)		-4.23 (-4.97, -3.48)		0.78 (-0.23, 1.80)	0.132
Medial tibial cartilage (% p.a.)	-3.29 (-4.28, -2.30)		-4.39 (-5.40, -3.39)		1.10 (-0.28, 2.48)	0.119
Lateral tibial cartilage (% p.a.)	-3.44 (-4.30, -2.58)		-4.09 (-4.96, -3.22)		0.65 (-0.55, 1.84)	0.290
Secondary Endpoints						
Tibiofemoral cartilage defects (0–16)	9.27 ± 3.10	9.70 ± 3.15	8.77 ± 2.94	9.17 ± 3.14	-0.17 (-0.43, 0.09)	0.208
Medial tibiofemoral defects (0–8)	4.87 ± 2.11	5.17 ± 2.13	4.65 ± 2.14	4.95 ± 2.16	-0.12 (-0.30, 0.06)	0.197
Lateral tibiofemoral defects (0–8)	4.40 ± 1.89	4.53 ± 1.87	4.12 ± 1.75	4.22 ± 1.87	-0.05 (-0.23, 0.13)	0.570
Tibiofemoral BMLs (0–39)	2.36 ± 2.95	2.29 ± 2.96	2.64 ± 2.88	2.89 ± 3.66	-0.45 (-0.92, 0.01)	0.059
Medial tibiofemoral lesions (0–18)	1.28 ± 2.24	1.21 ± 2.10	1.41 ± 2.26	1.51 ± 2.56	-0.22 (-0.54, 0.10)	0.186
Lateral tibiofemoral lesions (0–18)	0.83 ± 1.39	0.78 ± 1.41	0.91 ± 1.33	1.02 ± 1.56	-0.22 (-0.47, 0.03)	0.087
Post-hoc Endpoints						
VAS pain (0–100)	48.7 ± 21.4	33.7 ± 27.1	46.4 ± 20.5	36.4 ± 25.1	-5.4 (-10.7, -0.1)	0.048*
WOMAC total (0-2400)	687 ± 426	434 ± 419	665 ± 391	505 ± 436	-91.4 (-165.1, -17.7)	0.015*
Function (0-1700)	488 ± 318	306 ± 304	468 ± 293	362 ± 323	-72.9 (-126.4, -19.4)	0.008*
Stiffness (0-200)	61.5 ± 41.5	41.1 ± 44.1	61.7 ± 40.1	45.7 ± 41.1	-4.2 (-12.5, 4.0)	0.313

WOMAC, Western Ontario and McMaster University Index; VAS, visual analogue scale; p.a., per annum.
Between group difference is calculated using vitamin D group minus placebo group.
†WOMAC scores and VAS pain results are generated from mixed models adjusted for age, sex, body mass index, center and month.
‡Cartilage volume, cartilage defect and bone marrow lesion results are generated from multiple imputed datasets.
Higher scores in WOMAC, VAS pain, cartilage defects and bone marrow lesions indicate a more severe stage of the condition.
All analyses compare baseline versus 24 months. Asterisk signs indicate statistical significance.

6.3.5 Adverse events

Fifty-six participants in the vitamin D group reported at least one adverse event compared to 37 participants in the placebo group (Table 6.4). Four participants developed hypercalcemia in the vitamin D group, comparing to two in the placebo group. One participant in the vitamin D group had symptoms of hyperparathyroidism (e.g. muscle cramps, brittle bone and kidney dysfunction). There was one episode of renal calculus in each group.

Table 6.4: Adverse events.

	Vitamin D		Placebo	
	(N=209)	(%)	(N=204)	(%)
Serious Adverse Events				
Death	1	(0.5)	0	(0.0)
Malignancy	4	(1.9)	2	(1.0)
Coronary artery disease	1	(0.5)	1	(0.5)
Severe infection	0	(0.0)	3	(1.5)
Major depression	1	(0.5)	0	(0.0)
Nephrolithiasis	1	(0.5)	1	(0.5)
Hospitalization*	3	(1.4)	0	(0.0)
Adverse Events				
Hypercalcemia	4	(1.9)	2	(1.0)
Hyperparathyroidism	1	(0.5)	0	(0.0)
Renal	2	(1.0)	0	(0.0)
Falls	2	(1.0)	0	(0.0)
Musculoskeletal	1	(0.5)	1	(0.5)
Neurological	5	(2.4)	4	(2.0)
Gastrointestinal	7	(3.3)	5	(2.5)
Respiratory	2	(1.0)	2	(1.0)
Ocular	1	(0.5)	2	(1.0)
Infection	6	(2.9)	4	(2.0)
Cardiac arrhythmia	3	(1.4)	0	(0.0)
Chest pain	4	(1.9)	5	(2.5)
Pain	7	(3.3)	2	(1.0)
Allergy/immunology	0	(0.0)	2	(1.0)
Other eventsˆ	9	(4.3)	7	(3.4)

**Two were admitted to hospital after a fall and one was due to severe diarrhea.*
Încluding headache, lethargy, flu symptoms and other events.

6.4 Discussion

This RCT aimed to determine whether vitamin D supplementation could reduce knee pain and cartilage loss, and prevent progression of other knee structural abnormalities in knee OA patients with low 25OHD levels. Results showed that even in people with low 25OHD, supplementation did not slow cartilage loss or improve WOMAC assessed pain. These data suggest a lack of evidence to support vitamin D supplementation for slowing disease progression/structural change in knee OA.

Although epidemiological studies suggest that knee OA is more prevalent in those who are deficient in vitamin D, and vitamin D deficiency is associated with cartilage loss810 and knee OA symptoms [127, 128, 311], the results from two prior RCTs were mixed. In one study, supplementation of vitamin D3 2000 IU/day over two years showed no benefit for symptoms and structural changes in patients with knee OA, regardless of their 25OHD levels [129]. The other reported a small but statistically significant benefit on symptoms in patients with vitamin D insufficiency over one year [130]. Both studies have limitations. The first study included patients without vitamin D deficiency who may not benefit from vitamin D supplementation and patients whose disease was too severe to respond to vitamin D treatment. Also, it had a small sample size [305]. The second study did not examine structural changes and had a one-year follow-up, which may be too short to observe disease progression [312]. Our study addressed these limitations by recruiting patients without severe knee OA with low 25OHD levels and followed them for two years. Nonetheless, our results are largely consistent with the prior two trials.

Structural changes in cartilage and non-cartilaginous joint tissue assessed using MRI, are now recommended outcomes for clinical trials in OA [313]. An observational study showed that lower serum 25OHD levels were associated with greater cartilage volume loss over 2.7

years [120]. In the current study, the amount of tibial cartilage volume loss in the placebo group is consistent with the findings of a previous RCT [129]. We did not find significant effects on change in knee cartilage defects and bone marrow lesions.

Vitamin D side effects may include hypercalcemia. While intermittent very high dose vitamin D, for example 500,000 IU annually, may not be safe [314], our study suggests that the monthly regimen at a dosage of 50,000 IU is safe for the elderly, even though the serum 25OHD levels of long-term users are at the upper limit of the normal range [315].

The key strength of this RCT is the inclusion/exclusion criteria. We included only people with knee OA who had a vitamin D insufficiency, who may be the most likely to benefit from vitamin D supplements. We also used a predefined range of knee pain to prevent a ceiling or floor effect in the statistical analyses. We excluded patients with late stage knee OA, because there is very little cartilage left in these patients thus any possible benefits of therapy on cartilage would be difficult to identify. By using these criteria, we studied a patient population in whom the likelihood of demonstrating an effect (if truly present) of vitamin D supplementation was maximized.

The study also had limitations. First, WOMAC pain as a co-primary outcome was added during the recruitment period at the time the protocol was published. However, this change was made before the trial was completed, before any data analyses, and the original sample size had sufficient power to detect the expected difference in WOMAC pain. Second, loss to follow-up was 17.7% and was less in the vitamin D group (28 vs. 45 participants, $p = 0.021$). There were fewer participants who withdrew from their assigned intervention in the vitamin D group (8 vs. 21). Participants who did not comply with their assigned intervention could be expected to have a worse outcome than those who did. While this could bias the result towards the null, similar results were seen in per protocol analysis suggesting the differential drop out had minimal impact on our results. Last, we did not pre-specify clinical

outcomes such as visual analogue scale knee pain and WOMAC physical function as primary or secondary endpoints.

In conclusion, among patients with symptomatic knee OA and low serum 25OHD levels, vitamin D supplementation, compared with placebo, did not result in significant differences in change in MRI-measured tibial cartilage volume or change in WOMAC knee pain score over 2 years. These findings do not support the use of vitamin D supplementation for preventing tibial cartilage loss or improving WOMAC knee pain in patients with knee OA.

CHAPTER 7

Associations between Endogenous Sex Hormones and MRI Structural Changes in Patients with Symptomatic Knee Osteoarthritis

7.1 Introduction

The prevalence of knee OA in males and females is similar up to age 50 years, after which the prevalence for females increases more quickly than men [301, 316], suggesting a possible influence of the change in hormonal factors at the time of menopause on the development and progression of the disease. The relationship between hormonal changes due to menopause and OA was first described as early as 1926 and was referred to as 'menopausal arthritis' [317]. However, a clear causal relationship has yet to be established.

The available evidence for the relationship between sex hormones and knee structural changes in OA was systematically reviewed by Tanamas and colleagues in 2011 [318]. 27 studies were included in this review. However, due to the heterogeneity among the studies, there was insufficient evidence to draw any clear conclusion. One longitudinal study suggests an association between endogenous estrogen and radiographic OA in 842 premenopausal

and perimenopausal women [145]. One small cohort study of asymptomatic men showed a positive cross-sectional association between endogenous testosterone and tibial cartilage volume [319]; however, the longitudinal analysis found that testosterone was also associated with loss of cartilage volume over 2 years [320]. No significant relationship was found between testosterone and cartilage morphology or bone structure in an asymptomatic population [321].

The effects of endogenous sex hormones on knee OA structures remain uncertain, partly due to the lack of a longitudinal study with sufficient sample size in knee OA patients. Therefore, the aim of this study was to describe the longitudinal associations between serum levels of estrogen, progesterone and testosterone and knee structural changes using magnetic resonance imaging (MRI) in both males and females with symptomatic knee OA.

7.2 Method

7.2.1 Study design, setting and participants

This study included data on 200 participants who were randomly selected from the Vitamin D Effect on Osteoarthritis (VIDEO) study, which is described in Section 3.2. In the study, treatment and placebo groups were combined together as a cohort.

7.2.2 Assessment of pain

Knee pain was assessed at the baseline visit and at month 3, 6, 12 and 24 using a 100 mm visual analogue scale (VAS). Patients were asked to rate the severity of knee pain they experienced in the last month.

7.2.3 MRI assessment of knee structural changes

MRI scans of the study knee were obtained according to a standardized protocol as described in Section 3.6

Cartilage volume

Cartilage volume was determined using the previously described image processing techniques [120]. The volumes of individual cartilage plates (medial tibial and lateral tibial) were isolated by manually drawing disarticulation contours around the cartilage boundaries on a section-by-section basis then resampled by means of bilinear and cubic interpolation for final 3D rendering using OsiriX Lite imaging software (32-bit version 5.9, Pixmeo SARL, Geneva, Switzerland). The coefficient of variation (CV) was 2.1% for medial tibia and 2.2% for the lateral tibia [309].

Cartilage defects

Cartilage defects were graded using a modified Outerbridge classification [306] at medial tibial, medial femoral, lateral tibial and lateral femoral sites: grade 0, normal cartilage; grade 1, focal blistering and intracartilaginous hyperintensity with a normal contour; grade 2, irregularities on the surface and loss of thickness of less than 50%; grade 3, deep ulceration with loss of thickness of more than 50% without exposure of subchondral bone; grade 4, full-thickness chondral wear with exposure of subchondral bone. A total score was calculated as the total of subregional scores. Intra-observer reliability (expressed as ICC) was 0.90 for the medial tibiofemoral compartment, 0.89 for the lateral tibiofemoral compartment, 0.94 for the patellar compartment and 0.94 for the total score. Inter-observer 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, 0.93 for the patellar compartment and 0.93 for the total [177].

Bone marrow lesions

BMLs were defined as discrete areas of increased signal adjacent to the subcortical bone. The areas were measured semi-quantitatively using a modified Whole-Organ Magnetic Resonance Imaging Score (WORMS) [322] method in 15 sub-regions. The medial and lateral compartments of the tibial and femoral were divided into three sub-regions (anterior, central and posterior), and the tibia has one additional sub-region which represents the area beneath the tibial spines. Patellar was divided into medial and lateral sub-regions. BMLs were scored from 0 to 3 based on the extent of sub-regional involvement (0 = none; 1 = $<$25% of the sub-region; 2 = 25-50%; 3 = $>$50%). A total score (0-45) was calculated as the total of 15 sub-regional scores. The intra-observer reliability of this BML scoring system has been shown to be excellent [212, 323].

Effusion-synovitis volume

Effusion-synovitis volume was measured according to previously published methodology in the following sub-regions: 1) suprapatellar pouch, extends superiorly from the upper surface of the patellar, between the posterior suprapatellar fat pad (quadriceps femoris tendon) and the anterior surface of the femur; 2) central cavity, which includes the area between the central femoral and tibial condyles, around the ligaments and menisci, and the area behind the posterior portion of each femoral condyle, inside of the joint capsule. The effusion-synovitis volume was isolated by selecting a region of interest (ROI) with the intra-articular fluid-equivalent signal on a section-by-section basis. The total 3D volume was generated using OsiriX Lite imaging software. The intra-rater reliability (ICC = 0.96–0.97) and inter-

rater reliability (ICC = 0.93–0.99) were excellent.

7.2.4 Sex hormone assays

Fasting blood was drawn at baseline and two-year follow-up, and stored at -80C until assayed. Estradiol (E2) and progesterone (P) were measured using commercial enzyme-linked immunosorbent assay (ELISA) kits (Phoenix Pharmaceuticals Inc.; Burlingame, CA, USA). All assays were performed according to the manufacturers protocol. For estradiol, the assays Intra-class Correlation Coefficient (ICC) was 0.993, with a detection limit of 10 pg/ml. For progesterone, the ICC was 0.993, with a detection limit of 0.0625 ng/ml. Total testosterone (T) and sex hormone binding globulin (SHBG) was measured using a commercial ELISA kits (Demeditec Diagnostics GmbH, Kiel, Germany). For total T, the ICC was 0.997, with a detection limit of 0.083 ng/ml. For SHBG, the ICC was 0.994, with a detection limit of 0.2 nmol/L.

7.2.5 25OHD assays

Serum 25OHD was assayed by Liaison method utilizing a direct competitive chemiluminescent immunoassay (DiaSorin Inc., Stillwater, Minnesota, USA). The intra-assay and inter-assay coefficients of variation were 3.2% and 6.0%.

7.2.6 Statistical methods

Baseline characteristics were analyzed by descriptive statistics and were compared between males and females using Students t-test for continuous data and Chi-square test for dichotomous data.

Because of skewed distributions, serum sex hormone concentrations were categorized into

quartiles based on the distribution of values separately for males and females. The longitudinal associations between sex hormones and MRI structures were analyzed using a linear mixed-effects model. In the model, we entered terms for age, body mass index, sex hormone concentration, and vitamin D treatment allocation as fixed effects, and individual subject IDs as random intercepts. The analysis was performed separately for males and females due to sex differences in associations. The temporal relationship between sex hormone and MRI structures was further explored by adding the interaction term between sex hormone and age in the model.

7.3 Results

7.3.1 Participants

Table 7.1 shows the comparison of baseline characteristics between males and females. There was no significant difference in age, body mass index and serum vitamin D levels at baseline between sexes. Mean estradiol and progesterone levels were comparable between males and females, while testosterone levels were significantly higher for males and SHBG levels were significantly higher for females. There was no significant difference in VAS pain score. While males had more cartilage volume than females in both tibial and patellar compartments, the grades of cartilage defects and BMLs were similar between males and females. The volume of effusion-synovitis was greater in males than females.

7.3.2 Sex hormones and cartilage morphology

Table 7.2 shows the longitudinal associations between sex hormones and cartilage morphology. For males, sex hormone quartiles were not associated with total cartilage volume and cartilage defects. Similar results were observed for females, except for progesterone, where

Table 7.1: Baseline characteristics between males and females.

	Male (n = 107)	Female (n = 93)	P-value
Age (years)	63.9 ± 7.4	62.1 ± 7.2	0.10
BMI (kg/m^2)	29.0 ± 4.0	30.0 ± 5.6	0.16
25(OH)D (nmol/L)	44.4 ± 12.0	41.3 ± 12.2	0.08
Sex hormone profiles			
Estradiol (pg/ml)	28.9 ± 15.7	32.9 ± 26.5	0.19
Progesterone (ng/ml)	0.4 ± 0.2	0.5 ± 1.1	0.93
Testosterone (ng/ml)	3.5 ± 1.5	0.4 ± 0.4	<0.01*
SHBG (nmol/L)	34.0 ± 15.3	52.3 ± 27.8	<0.01*
Clinical symptoms			
VAS pain (0–100)	45.8 ± 19.8	49.2 ± 20.6	0.24
MRI structures			
Tibial cartilage volume (mm^3)	4.3 ± 1.0	3.0 ± 0.6	<0.01*
Patellar cartilage volume (mm^3)	2.8 ± 0.8	1.7 ± 0.6	<0.01*
Total cartilage defects (0–24)	14.2 ± 4.0	14.7 ± 4.4	0.47
Total BMLs (0–45)	3.7 ± 3.7	3.4 ± 3.0	0.5
Total effusion volume (mm^3)	12.3 ± 10.8	7.7 ± 7.0	<0.01*
Total effusion grade (0–3)	2.1 ± 0.9	1.6 ± 0.9	<0.01*

BMI, body mass index; SHBG, sex hormone binding globulin;
Results are shown as mean (standard deviation).
Unpaired student t-test was used for the comparison.
** indicates statistical significance at α = 0.05.*

higher progesterone quartiles were significantly associated with more cartilage volume (β = 0.12 per quartile, p = 0.01). There were no statistically significant interactions between any sex hormones and age.

7.3.3 Sex hormones, BMLs and effusion-synovitis

Table 7.3 shows the longitudinal relationships of sex hormones quartiles with BMLs and effusion-synovitis. No statistically significant relationship was observed for males. In contrast for females, higher quartiles of estradiol levels were associated with a lower grade of BMLs (β = -0.45 per quartile, p = 0.03), but there were no associations between BML grade and progesterone, testosterone or SHBG levels. Higher quartiles of estradiol (β = -1.26 per quartile, p = 0.05), progesterone (β = -1.60 per quartile, p <0.01) and testosterone (β = -

Table 7.2: Linear mixed-effect model for association between sex hormones and cartilage morphology over 2 years.

	Male (n = 107)		Female (n = 93)	
	β (95% CI)†	P-value	β (95% CI)†	P-value
Total cartilage volume (mm^3)				
Estradiol	-0.06 (-0.22, 0.10)	0.48	-0.07 (-0.17, 0.04)	0.20
Progesterone	0.13 (0.00, 0.27)	0.06	0.12 (0.03, 0.21)	0.01*
Testosterone	0.05 (-0.09, 0.20)	0.47	-0.02 (-0.12, 0.07)	0.65
SHBG	0.06 (-0.11, 0.24)	0.49	0.08 (-0.04, 0.20)	0.18
Total cartilage defects (0-24)				
Estradiol	-0.22 (-0.62, 0.18)	0.28	0.02 (-0.39, 0.43)	0.93
Progesterone	0.03 (-0.32, 0.37)	0.89	0.21 (-0.15, 0.57)	0.26
Testosterone	-0.02 (-0.38, 0.34)	0.92	-0.14 (-0.51, 0.23)	0.45
SHBG	0.21 (-0.23, 0.66)	0.35	0.30 (-0.17, 0.77)	0.21

SHBG, sex hormone binding globulin; CI, confidence interval.
†Multivariable analysis adjusted for age, body mass index and randomization.
Beta coefficient represents change in cartilage per quartile increase in corresponding sex hormone.
** indicates statistical significance at α = 0.05.*

Table 7.3: Linear mixed-effect model for association between sex hormones and bone marrow lesions and effusion-synovitis over 2 years.

	Male (n = 107)		Female (n = 93)	
	β (95% CI)†	p-value	β (95% CI)†	p-value
Total BML (0-45)				
Estradiol	-0.13 (-0.63, 0.37)	0.62	-0.45 (-0.86, -0.05)	0.03
Progesterone	0.03 (-0.45, 0.50)	0.9	-0.19 (-0.58, 0.19)	0.32
Testosterone	0.12 (-0.38, 0.62)	0.64	-0.20 (-0.58, 0.19)	0.32
SHBG	0.21 (-0.34, 0.76)	0.45	-0.04 (-0.50, 0.41)	0.85
Effusion volume (mm^3)				
Estradiol	-0.73 (-2.33, 0.86)	0.37	-1.26 (-2.50, -0.02)	0.05
Progesterone	0.86 (-0.61, 2.34)	0.25	-1.60 (-2.77, -0.43)	<0.01
Testosterone	-0.13 (-1.67, 1.42)	0.87	-1.49 (-2.71, -0.27)	0.02
SHBG	0.90 (-0.84, 2.65)	0.31	-0.25 (-1.62, 1.12)	0.72

BML, bone marrow lesion; SHBG, sex hormone binding globulin; CI, confidence interval.
†Multivariable analysis adjusted for age, body mass index and randomization.
Beta coefficient represents change in BML or effusion volume per quartile increase in corresponding sex hormone.
Bold values indicate statistical significance at α = 0.05.

1.49 per quartile, p = 0.02) were associated with less effusion-synovitis volume for females. The levels of SHBG were not associated with effusion-synovitis. There were no statistically significant interactions between any sex hormones and age.

7.3.4 Sex hormones and VAS pain

Table 7.4 shows the longitudinal association of sex hormones quartiles with knee VAS pain. For males, none of the sex hormones was associated with VAS pain score. In contrast, higher quartiles in testosterone were associated with a lower VAS pain for females (β = -3.90 per quartile, p = 0.02). Neither estradiol nor progesterone in females were significantly associated with VAS pain score.

Table 7.4: Linear mixed-effect model for association between sex hormones and VAS pain over 2 years.

	Male (n = 107)		Female (n = 93)	
	β (95% CI)†	p-value	β (95% CI)†	p-value
VAS pain (0-100)				
Estradiol	2.26 (-0.68, 5.19)	0.13	-1.61 (-4.92, 1.70)	0.26
Progesterone	0.54 (-2.40, 3.47)	0.93	-1.05 (-4.37, 2.27)	0.54
Testosterone	0.13 (-2.94, 3.20)	0.93	-3.90 (-7.12, -0.68)	0.02
SHBG	2.97 (-0.18, 6.12)	0.06	-1.93 (-5.47, 1.60)	0.28

SHBG, sex hormone binding globulin; CI, confidence interval.
†Multivariable analysis adjusted for age, body mass index and randomization.
Beta coefficient represents change in VAS per quartile increase in corresponding sex hormone.
Bold values indicate statistical significance at α = 0.05.

7.4 Discussion

Our study is the first to examine the longitudinal associations between endogenous sex hormone levels and different MRI-assessed joint structures in both males and females with symptomatic knee OA. Our hypothesis was that sex hormone deficiency was associated with OA-related joint structural abnormalities, and this was supported by our data in females but

not in males. We found no associations between endogenous sex hormones and joint structural changes measured as cartilage volume, cartilage defects, BMLs and effusion-synovitis in male patients with knee OA. However, in females, progesterone was positively associated with cartilage volume, estradiol was negatively associated with BMLs, and all sex hormones had inverse relationships with total effusion-synovitis volume. The discrepancy in the effect of sex hormones between males and females may be attributable to the fact that gene expression patterns for sex hormone receptors differed by sex and the regulatory effect of sex hormones on collagen gene expression in the cartilage was different among males and females [324].

MRI provides higher sensitivity than radiograph in detecting early structural changes and has become a popular tool for OA research. Measurement of cartilage morphology, BMLs and effusion-synovitis has been found associated with the progression of knee OA [325]. There are very few epidemiological studies examining associations between endogenous sex hormones and MRI structures in the symptomatic knee OA population. In a small 2-year cohort study of 28 healthy men aged 51.9 years, Hanna and colleagues found that increased serum testosterone was associated with greater cartilage volume in a cross-sectional analysis, while higher serum free testosterone was associated with increased tibial cartilage volume loss over 2 years. The authors suggested that the benefit of testosterone on cartilage volume was only temporary and that the longitudinal inverse association may be due to a higher level of physical activity which results in greater biomechanical stress in the articular cartilage [319]. However, in another cross-sectional study conducted by the same research group, no associations were found between testosterone and cartilage morphology or bone structures in 139 asymptomatic midlife females [321]. No significant relationship was found between knee estrogen and knee structure [319, 320, 321]. Our findings were similar to these studies as no significant associations were observed in between estradiol, testosterone and total cartilage volume in both males and females. Interestingly, we found progesterone was positively

associated with cartilage volume for females. Progesterone is the precursor for all steroid hormones and its receptors have been localized in the chondrocytes of knee cartilage [326]. Progesterone may play a role in the maintenance of cartilage volume by suppressing the production of matrix metalloproteinases (MMPs) family [327], which are primarily responsible for the degradation of articular cartilage. In addition, combined therapy with estrogen and progesterone was found to be more effective in suppressing cartilage turnover compare to estrogen alone treatment in a murine model of OA [328].

The fact that the prevalence of knee OA in women increases dramatically in the years after menopause suggest that the drop in sex hormones at the time of menopause increase the risk getting knee OA in women. Urinary cross-linking telopeptide of type II collagen (uCTX-II) is a prognostic biomarker candidate for knee OA [329] and its levels have been found to increase after menopause, consistent with the acceleration of knee OA in postmenopausal women [330]. uCTX-II has unique relations with bone metabolism [331] and Garnero and colleagues found that BMLs on MRI were significantly associated with uCTX-II [332]. It is well established that estrogen plays a major role in regulating bone turnover and remodeling by modulating the activity of osteoblasts and osteoclasts [333]. The findings of the present study confirm the link between estrogen, bone metabolism and OA-related subchondral bone abnormalities as higher estradiol levels in women were observed to be associated with lower total BML scores. This result is consistent with previous findings where postmenopausal women taking estrogen replacement therapy showed a significantly decreased prevalence of BMLs compared with those not taking it [334].

Joint swelling is one clinical feature of knee OA reflecting the presence of effusion- synovitis on MRI. Low-grade inflammation and synovitis-effusion have been strongly implicated in the pathogenesis of knee OA [237, 83]. It has been known for many decades that sex hormones are involved in the modulation of immune responses [335]. Estrogen and progesterone receptors were found in the lining cells of human synovium [326, 336]. While

progesterone and testosterone are thought to be anti-inflammatory [337, 338], estradiol has been demonstrated to have pro-inflammatory effects at high doses, but anti-inflammatory properties via inhibition of IL-1β at low doses [339]. Our results show that estradiol, progesterone and testosterone were all negatively associated with effusion-synovitis volume for females. The inverse relationship between estradiol and effusion-synovitis volume may be a result of the anti-inflammatory effects of low dose estrogen which is characteristic in this postmenopausal population. Circulating testosterone gradually decrease in postmenopausal women and the low testosterone levels have been linked to increased pain sensitivity [340]. In this study, we also found that higher levels of testosterone for females were associated with lower VAS pain score.

The interaction terms between sex hormones and age were added in the mixed-effect model to explore the temporal relationship between sex hormones and MRI structures. The interaction terms appear to be statistically non-significant in our analyses, suggesting that the strength of the association may not change over time. Although this study was unable to show that changes in sex hormones preceded MRI structural changes, it is biologically unlikely that MRI structural changes could cause alteration of sex hormone levels.

There were several potential limitations. This study is conducted as a post-hoc analysis within a subsample of an RCT, and the results applied to the population sample of the original study, which is in the context of symptomatic knee OA with vitamin D insufficiency, but may not generalizable to the general population of knee OA. While allocation to vitamin D treatment may intervene in the relationship between sex hormones and MRI structures, repeated analyses adjusted for vitamin D levels yield very similar results (data not shown). Another limitation of this study is that information regarding previous reproductive histories and concurrent use of hormone replacement therapy was not obtained during the clinical visit because the original RCT was not designed to study the effects of sex hormones in knee OA patients. Also, we did not select the timing of blood sample collection for sex

hormone testing, as blood was collected at each clinical visit scheduled based on the original RCT. However, the women in this study were to be postmenopausal due to age, so their sex hormones should not fluctuate as they do in premenopausal women. Even if fluctuation did occur in women, the increased variance in hormones would lead to bias towards the null, therefore, our findings would not be expected to change due to hormonal fluctuation.

There are a number of strengths in our study. First, it examines the sex hormone levels in a symptomatic knee OA population, in comparison to previous literature where sex hormones are examined in asymptomatic healthy population. Second, we utilized state-of-the-art MRI scan to analyze multiple structural changes in the knee as a whole. Lastly, our study is a prospective longitudinal analysis with sufficiently large sample size to establish any relationships between sex hormones and the knee structures.

In conclusion, endogenous estradiol, progesterone and testosterone may be protective for joint structural changes in women but not men. This may contribute to observed sex differences in knee OA.

CHAPTER 8

MRI Markers and the Prediction of Total Knee Replacement

8.1 Introduction

Magnetic Resonance Imaging (MRI) allows a non-invasive, three-dimensional assessment of the entire joint and is increasingly used in knee OA research. MRI provides higher sensitivity than x-ray in detecting early knee OA with a semi-quantitative assessment of cartilage defect, bone marrow lesion (BML), effusion-synovitis, and meniscal pathology [341, 342]. These structural pathologies are associated with the clinical and structural progression of knee OA [325, 343, 344, 345].

Total knee replacement (TKR) is an important clinical endpoint for end-stage knee OA. TKR is considered when knee OA patients have progressively increasing pain and decreasing physical function. During 2014, over 54,277 knee replacements were performed in Australia with each procedure costing between $15,000 to $31,900 [346]. The incidence of TKR for knee OA is rising steeply, and expected to increase by more than 6-fold in the US by 2030 [347]. MRI could detect early structural abnormalities on MRI and therefore identify potential targets for effective treatment, which may ultimately lower or delay the need for

TKR in the long term.

The European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO) suggested MRI as a viable alternative to radiography for prediction of TKR in a literature review [348]. One study of 117 patients with knee OA reported that high cartilage defect scores were associated with a six-fold increased risk of TKR over 4 years compared with those with lower scores [186]. In another longitudinal study of 109 patients with knee OA, severity of BMLs was associated with increased risk of TKR over 4 years [214]. The risk for TKR was significantly increased in knees with MRI abnormalities, such as more than two subregions with severe cartilage loss, more than two subregions with BMLs, medial meniscal maceration but not extrusion, and effusion or synovitis [215]. However, the independent predictive value of each structural pathology has not been examined in these studies. Also, the clinical implication of these studies is limited by their relatively small sample size and short follow-up time, and most of the studies examined participants with definite knee OA, which has very high rates of knee replacement ($>10\%$), thus the data may not be applicable to the general population. Therefore, the independent predictive validity of MRI structural pathologies for TKR needs to be further examined in a large community-based cohort with longer-term follow-up.

Previous data from our community-based study showed that cartilage defects [178] and BMLs [199] predicted risk of TKR over 5 years but there were relatively few TKRs. The aim of this study was to examine whether cartilage defects, BMLs, meniscal tear, meniscal extrusion and effusion-synovitis at baseline predict long-term TKR over 10 years.

8.2 Methods

8.2.1 Study design, setting and participants

This study used the data from the Tasmanian Older Adult Cohort Study (TasOAC) as described in Section 3.1.

8.2.2 MRI scans

The full details of MRI specifications are described in Section 3.6

Cartilage defects

Cartilage defects were assessed on T1-weighted MRI and graded at medial tibial, medial femoral, lateral tibial, lateral femoral and patellar regions using a modified Outerbridge system: grade 0, normal cartilage; grade 1, focal blistering and low-signal intensity change 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 loss with exposure of subchondral bone [177]. A cartilage defect also had to be present in at least two consecutive slices. The total cartilage defect score was the highest score of all compartments of the knee. Intra-observer reliability (expressed as ICC) was 0.89–0.94 and inter-observer reliability was 0.85–0.93 [177].

Bone marrow lesions

Bone marrow lesions (BMLs) were defined as poorly marginated areas of increased signal adjacent to the subcortical bone on T2-weighted MRI and scored at medial tibial, medial femoral, lateral tibial, lateral femoral and patellar regions using a modified version of Whole-

Organ Magnetic Resonance Imaging Score (WORMS): grade 0, absence of BML; grade 1, area smaller than 25% of the region; grade 2, area between 25% to 50% of the region; grade 3, area larger than 50% of the region [322]. The total BML score was the highest score of the region out of all the regions. The inter-reader reliability of this BML scoring system has been shown to be excellent [212, 323].

Effusion-synovitis

Effusion-synovitis was assessed on sagittal T2-weighted MRI and graded using a modified version of WORMS at suprapatellar pouch, central portion, posterior femoral recess and subpopliteal recess. Grading from 0 to 3 was based on the maximal estimated distention of the synovial cavity: grade 0, normal; grade 1, $<$33% of maximum potential distention; grade 2 to 33%–66% of maximum potential distention; grade 3, $>$66% of maximum potential distention [16]. The intra-rater reliability (expressed as weighted) was 0.63–0.75 in different subregions and the inter-rater reliability was 0.65–0.79 [225].

Meniscal pathologies

Meniscal abnormalities, including meniscal tear and extrusion, were assessed on T1-weighted MRI. Each meniscus was divided into three areas (anterior horn, body and posterior horn). For meniscal tears, the following scale was applied: 0 = no damage; 1 = one of three meniscal areas involved; 2 = two of three areas involved; 3 = all three areas involved [349]. Extrusion was scored on a scale from 0 to 2: grade 0, no extrusion; grade 1 (partial extrusion), meniscal tissue extends beyond the tibial margin; and grade 2 (complete extrusion), the meniscus has no contact with the joint space [350]. The highest score of both medial and lateral menisci was used for analysis.

8.2.3 Knee replacement surgery

At the follow-up visits, participants were asked whether they had undergone a TKR since their first visit. Although MRI scans were taken on the right knee only at baseline, knee replacement data were collected on both knees.

8.2.4 Radiographs

The specifications for knee radiographs is detailed in Section 3.5

8.2.5 WOMAC score

Knee pain at baseline was assessed using the Western Ontario and McMaster Universities Osteoarthritis (WOMAC) score, which is a self-administered questionnaire consists of 3 subscales and 24 items (5 pain, 2 stiffness and 17 function items) [230]. We used a 10-point Likert version of WOMAC from 0 (no pain, stiffness or function deficit) to 9 (most severe pain, stiffness or severe function problems). A total WOMAC score was generated by summing up all the items.

8.2.6 Data analysis

T-tests or chi-square tests were used to assess the differences between groups of participants who underwent TKR and those who did not. The association between baseline cartilage defects, BMLs, effusion-synovitis, meniscal tear and meniscal extrusion and the risk of TKR was measured with a hazard ratio (HR) using a Cox proportional hazards model after adjustment for baseline WOMAC pain score and ROA, which are the two main factors that predict TKR in clinical practice. The model was further adjusted for other MRI structural

pathologies to evaluate the independent association of each structural feature.

8.3 Results

8.3.1 Characteristics of the study participants

A total of 972 subjects had all structural markers measured on baseline knee MRI scans. Over the course of 10.7 years, a total of 37 participants (3%) reported 43 TKRs, including 23 on the same knee of the MRI scan and 20 on the opposite knee. Table 8.1 shows the differences in the baseline characteristics between study participants who had a TKR compared to those who did not. Participants who reported a TKR had a higher mean BMI, higher WOMAC scores, and higher prevalence of cartilage defects, BMLs and effusion-synovitis. While participants who had TKR all had meniscal tears, there was no difference in meniscal extrusion between those who had TKR and those did not.

Table 8.1: Baseline characteristics of participants.

	No knee replacement (n=935)	Knee replacement (n=37)	P value†
Age (years)	62.8 ± 7.4	63.9 ± 7.7	0.35
Female (%)	50%	59%	0.25
BMI (kg/m^2)	27.6 ± 4.6	29.4 ± 5.6	0.02
ROA (%)	59%	74%	0.09
WOMAC score	15.1 ± 27.5	46.5 ± 41.3	<0.01
Pain	3.3 ± 6.0	9.3 ± 7.5	<0.01
Stiffness	1.5 ± 2.7	4.1 ± 3.9	<0.01
Function	10.2 ± 19.9	33.2 ± 32.6	<0.01
Cartilage defects (%)	51%	84%	<0.01
BMLs (%)	43%	73%	<0.01
Effusion-synovitis (%)	22%	43%	<0.01
Meniscal extrusion (%)	4%	4%	0.93
Meniscal tear (%)	81%	100%	0.01

BMI, body mass index; ROA, radiographic osteoarthritis; BMLs, bone marrow lesions;
WOMAC, Western Ontario and McMaster Universities Osteoarthritis Score;
Data reported as the mean ± standard deviation, except where indicated.
†By student t-test or chi-square test; bold values indicate statistical significance at $\alpha = 0.05$

Table 8.2: Association between baseline MRI structural markers and total TKR over 10.7 years.

	Multivariable 1*		Multivariable 2†	
	HR	95% CI	HR	95% CI
Cartilage defects	2.32	(1.67, 3.21)	1.84	(1.21, 2.80)
Grade 0/1		Ref.		
Grade 2	2.31	(0.70, 7.61)	1.24	(0.33, 4.64)
Grade 3	8.61	(3.07, 24.14)	3.98	(1.24, 12.77)
Grade 4	10.95	(3.50, 34.27)	4.77	(1.21, 18.80)
Bone marrow lesions	2.00	(1.49, 2.68)	1.48	(1.00, 2.18)
Grade 0		Ref.		
Grade 1	0.99	(0.30, 3.22)	0.47	(0.11, 1.98)
Grade 2	5.51	(2.17, 14.00)	2.47	(0.76, 8.02)
Grade 3	6.42	(2.61, 15.81)	2.11	(0.63, 7.12)
Effusion-synovitis‡	2.41	(1.21, 4.81)	1.56	(0.66, 3.68)
Grade 0/1		Ref.		
Grade 2	0.92	(0.37, 2.29)	0.58	(0.19, 1.76)
Grade 3	2.29	(0.96, 5.47)	1.09	(0.37, 3.25)
Meniscal extrusion	1.40	(0.68, 2.86)	1.01	(0.51, 2.00)
Grade 0		Ref.		
Grade 1	1.47	(0.60, 3.61)	1.37	(0.55, 3.41)
Grade 2	1.73	(0.22, 13.45)	0.57	(0.07, 4.65)

TKR, total knee replacement; HR, hazard ratio; CI, confidence interval.
** Adjusted for radiographic osteoarthritis and baseline pain.*
†Further adjusted for other MRI structural pathologies.
‡Comparison of grade 3 effusion-synovitis against the rest of the cohort.
Bold values indicate p value <0.05.

8.3.2 Association between baseline MRI structural markers and TKR

The associations between MRI structural markers (cartilage defects, BMLs, effusion-synovitis and meniscal extrusion) and TKR are described in Table 8.2. After adjustment for baseline WOMAC pain score and ROA, cartilage defects, BMLs and grade 3 effusion-synovitis at baseline were statistically significantly associated with higher hazard for TKR. In subregional analysis, grade 3 effusion-synovitis in the suprapatellar pouch was significantly associated with TKR (HR=3.96, 95% CI 1.94–8.09). Both cartilage defects and BML grades

showed a dose-response association with risk of TKR, while only grade 3 effusion-synovitis showed a significantly increased risk of TKR (Figure 8.1). The associations for cartilage defects remained statistically significant after further adjustment for other MRI structural markers. The association for BMLs became weaker but remained statistically significant after further adjustment for other MRI structural changes. Effusion-synovitis was not associated with TKR after further adjustment. Meniscal extrusion was not associated with the risk of TKR over 10.7 years.

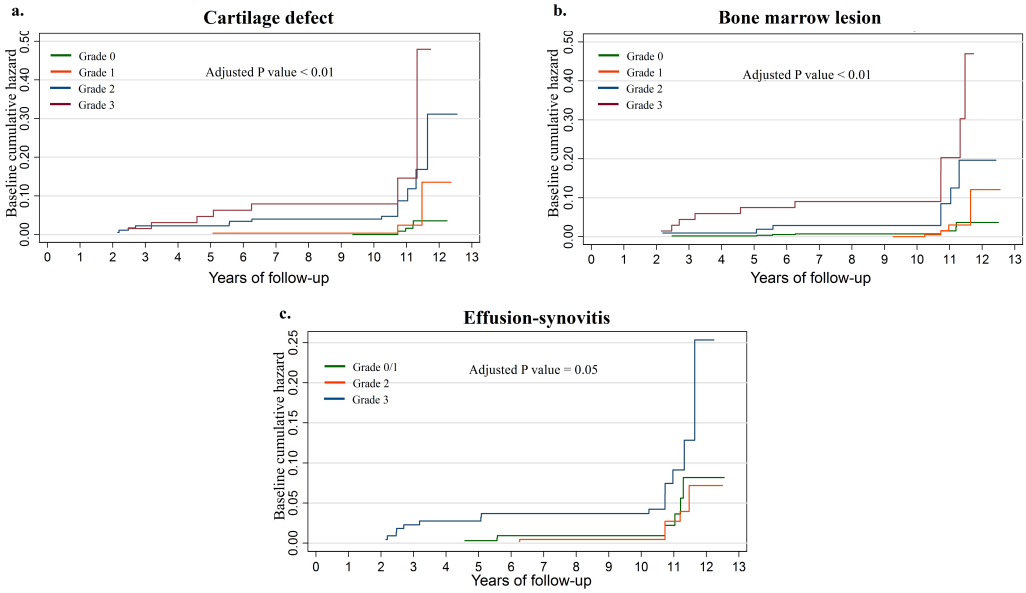


Figure 8.1: Cumulative hazard of total knee replacement stratified by cartilage defects (a), bone marrow lesion (b) and effusion-synovitis (c).

8.3.3 Association between baseline number of MRI pathologies and TKR

Table 8.3 describes the associations between the total number of MRI pathologies present at baseline and TKR over 10.7 years. The risk of TKR increased significantly when more

MRI pathologies were simultaneously present at baseline (HR=1.84/unit, 95%CI 1.35–2.51). Interestingly, baseline number of MRI pathology present in the right knee was associated with the risk of TKR on the same knee (HR=1.80/unit, 95% CI 1.23–2.64), and the risk of TKR on the opposite knee (HR=1.66/unit, 95% CI 1.09–2.52). There was also a dose-response relationship between the number of MRI pathologies at baseline and the risk of TKR (Figure 8.2).

Table 8.3: Total number of baseline MRI pathology and knee replacement over 10.7 years.

Number of pathologies	Right knee		Left knee		Total†	
	Count	%	Count	%	Count	%
0 (n = 105)	0	0.0%	0	0.0%	0	0.0%
1 (n = 307)	1	0.3%	5	1.6%	5	1.6%
2 (n = 262)	7	2.7%	3	1.1%	8	3.1%
3 (n = 219)	11	5.0%	8	3.7%	16	7.3%
4–5 (n = 95)	4	4.2%	4	4.2%	8	8.4%
Hazard ratio (95% CI)*	1.8	(1.23, 2.64)	1.66	(1.09, 2.52)	1.84	(1.35, 2.51)

†Total number of patients who underwent either right or left knee replacement.
** Adjusted for radiographic osteoarthritis and baseline pain.*
Bold values indicate p<0.05.

8.4 Discussion

To our knowledge, this study is the first longitudinal population-based study to describe the long-term associations between cartilage defects, BMLs, meniscal pathologies and effusion-synovitis and TKR in older adults over 10.7 years. Our data showed that baseline knee cartilage defects, BML, meniscal tear but not grade 3 effusion-synovitis or meniscal extrusion were associated with an increased risk of TKR. In addition, the number of pathologies present on MRI predicted the occurrence of TKR over 10.7 years.

This study demonstrates a dose-response and independent relationship between baseline cartilage defect grade and the risk of TKR. The cumulative risks for TKR increased with higher grades of cartilage defects at baseline, and the risk reached nearly 50% in those with grade

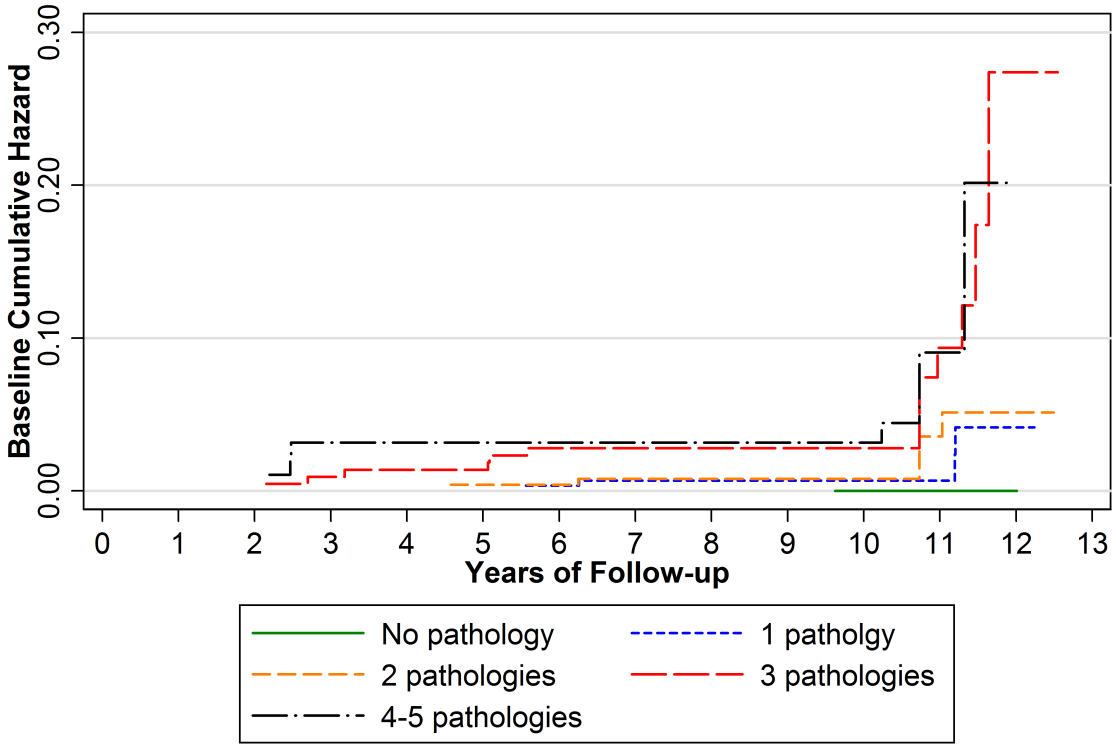


Figure 8.2: Cumulative hazard of total knee replacement stratified by number of MRI pathologies.

4 defects after 10 years. This is similar to our previous published data in the same cohort where higher cartilage defect grade at baseline predicted higher risk of TKR over 5 years [178]. The relationship is also supported by another study which showed that the severity of tibiofemoral cartilage defects at baseline was an independent risk factor for TKR over 4 years [186]. They found that the higher cartilage defect scores were associated with higher risk for surgery in comparison to lower scores.

BML is associated with clinical and structural progression of knee OA and is increasingly suggested by researchers as an outcome measure in clinical trials [351]. We found that baseline BMLs were an independent predictor of TKR after adjustment for all other structural pathologies. This is consistent with our previous data showing that the severity of BMLs was

a strong predictor of TKR over 5 years after adjustment for some structural factors [199]. In addition, a follow-up study of the randomized controlled trial (RCT) of knee OA population found that medial BML (OR=1.48, 95% CI 1.21–1.82) was one of the strongest predictors of TKR [175]. Similarly, different studies have shown that BMLs in knee OA predicted TKR in the following year [215], within three years [213] and after four years [214].

Meniscal pathology is very common in older population [352] and is associated with structural and clinical progression of knee OA [352, 353]. Baseline severe medial meniscal tear is one of the strongest independent long-term predictors of TKR in knee OA patients [175]. In our study population, 82% of participants had some degree of meniscal tear and we found that all the participants who underwent TKRs had a meniscal tear at baseline. This finding suggests the importance of a severe meniscal tear in the progression of knee OA leading to TKR. However, meniscal extrusion was not associated with TKR in this population-based sample. This could be due to the very low prevalence of extrusion in this population (4%) and the number of TKRs in this group was too small to detect a statistical significance. However, similar results were observed in a study where the risk for TKR was significantly increased for knees that exhibited two or more subregions with MRI structural pathologies except extrusion [215].

Recent evidence suggests the involvement of effusion-synovitis in the progression of knee OA [225, 85] and its role as a treatment target for knee OA [354]. However, there is very few data on the predictive validity of effusion-synovitis on TKR. Roemer et al suggest that the risk for TKR was significantly increased for knees that exhibited two or more subregions with effusion-synovitis, when compared with knees that did not exhibit these features [215]. The present study found that the association between total effusion-synovitis and TKR was only significant for grade 3 effusion-synovitis, but this did not persist after adjustment for the other factors. This suggests the association is confounded by the other pathologies or on the causal pathway between these pathologies and TKR. If it is on the casual pathway, then

effusion-synovitis remains a valid target in OA trials.

A recent study suggested that all MRI tissue abnormalities, except for meniscal extrusion could be used to predict TKR [215]. This leads us to question whether there is incremental risk for TKR when multiple MRI structural abnormalities are present. This study is the first to show that the number of MRI pathologies present at baseline significantly predicted the occurrence of TKR over 10 years in a dose-response manner. On average, the risk of having a TKR increased by 84% for each structural abnormality present. The risk of having a TKR in those who had 4-5 structural abnormalities could be as high as 5.25 times of that in those who had only one abnormality.

The major strength of our study is that it is a prospective population-based study with a large sample size and a long follow-up period. Our results have good external validity and are generalizable to all older adults in the population. Our study utilizes all the data from multiple clinical visits throughout the 10.7 years of follow-up, adding extra validity and reliability of our findings. We also acknowledge that there are some limitations in our study. Cartilage defects were assessed on T1-weighted GRE MR images and some may argue that GRE sequences are less suited for cartilage defect measurement [355]. However, there is evidence that GRE sequences have high sensitivity and specificity detecting cartilage defects compared to observations under arthroscopy [356, 357]. The retention rate in our study after 10.7 years was 67%. Participants who were lost to follow-up were older and had more cartilage defects and higher WOMAC scores at the baseline compared to those who were followed up (data not shown). It is most likely that the loss of those with more severe disease may underestimate our findings.

In conclusion, cartilage defects, BMLs and meniscal tears, but not effusion or meniscal extrusion in the right knee were independent predictors of TKR in either knee over 10.7 years. Presence of multiple pathologies increased the risk of TKR, suggesting that MRI structural

markers are good predictors of rapid knee OA progression in the general population.

CHAPTER 9

Summary and Future Directions

9.1 Summary

OA is the most common joint disease in the world and it is one of the most frequent causes of pain, loss of function, and disability in Western populations [301]. The knee is the most common joint affected by OA and pain is the prominent symptom. In Australia, it was estimated to over $1.8 million or 8.0% Australians was affected by OA [3]. The disease was one of the most expensive diseases in Australia, costing $1.6 billion (2.5%) of the total health care expenditure each year in Australia. With an aging population and a rising obesity rate, the social and economic burden associated with OA is increasing. Despite its heavy socioeconomic burden, there are no proven preventative strategies and no registered effective treatments which stop or delay the progression of the disease. Conventional treatment options for knee OA are mostly palliative and costly. There is an urgent need for identifying modifiable risk factors and developing a cost-effective treatment for this disease. Recently, knee OA is recognized as a disease of the whole joint with multiple structural abnormalities. MRI is becoming a popular tool in knee OA research for the visualization of all structures within in the joint. This thesis has examined the roles of systemic risk factors (low-grade inflammation, adiposity and sex hormones) in disease progression and the predictive values

of MRI markers for knee replacement, as well as the effects of vitamin D supplementation on the disease. Several novel and important findings presented in this thesis are summarized below.

Chapter 4 systematically reviews 32 relevant studies for the relationship between serum CRP levels measured by a high sensitivity method (hs-CRP) and OA, as well as the correlation between circulating CRP levels and OA phenotypes. Serum hs-CRP levels in OA were modestly but statistically significantly higher than controls (mean difference = 1.19 mg/l, 95% CI 0.64 to 1.73). The levels were significantly associated with pain (r = 0.14, 95% CI 0.09 to 0.20, $p<0.001$) and decreased physical function (r = 0.25, 95% CI 0.13 to 0.39). No significant associations were found between hs-CRP levels and radiographic OA. This suggests low-grade systemic inflammation may contribute to the pathogenesis of OA. The role of low-grade systemic inflammation may be greater in causing symptoms rather than radiographic changes in OA.

Adipose tissue is a dynamic endocrine organ that secretes a number of factors that are increasingly recognized to contribute to systemic inflammation [358]. Chapter 5 describes the longitudinal relationship between adiposity and change in knee pain in a population-based sample over 5.1 years. Baseline BMI and body fat mass were deleteriously associated with consistent knee pain over follow-up. BMI was consistently associated with increases in weight-bearing and non-weight-bearing pain. Fat mass was associated with an increase in non-weight-bearing pain. In mixed-model analyses, WOMAC total pain score was associated with BMI (beta = 1.27, 95% CI 0.89 to 1.66) and body fat mass (beta = 1.17, 95% 0.76 to 1.59).The association of lean mass was not significant after adjustment for fat mass. These results show that BMI is the most consistent correlate of knee pain. Fat mass is associated with non-weight-bearing knee pain suggesting systemic and metabolic mechanisms are involved.

Observational studies suggest vitamin D supplementation as a potential cost-effect treatment for knee OA [304]. Chapter 6 compares the effects of vitamin D supplementation versus placebo on knee pain and knee cartilage volume in 413 patients with symptomatic knee OA and low vitamin D levels in a multi-center randomized clinical trial. Monthly 50,000IU vitamin D supplementation, compared with placebo, did not result in significant differences in change in MRI-measured tibial cartilage volume (-3.44% versus -4.23%, p = 0.132) or WOMAC knee pain score (-49.9 versus -35.1, p = 0.102) over 2 years. Although the vitamin D group had greater improvement in VAS knee pain (-15.4 mm vs. -8.9 mm, p = 0.048) in the secondary analyses, there were no significant differences in changes of tibiofemoral cartilage defects or BMLs. There are slightly more participants in the vitamin D group than in the placebo group (56 versus 37) who had at least one adverse event. These findings do not support the use of vitamin D supplementation for preventing tibial cartilage loss or improving WOMAC knee pain in patients with knee OA.

Chapter 7 describes the longitudinal associations between endogenous sex hormones and MRI knee structural changes in 107 men and 93 women with symptomatic knee OA. For women, after adjustment for confounding factors, progesterone was associated with cartilage volume(β=0.12/quarter, 95% CI 0.03 to 0.21)and estradiol levels were associated with lower grades of BMLs (β=-0.45/quarter, 95% CI -0.86 to -0.05), while estradiol (β=-1.26/quarter, 95% CI -2.50 to -0.02), progesterone (β=-1.60/quarter, 95% CI -2.77 to -0.43) and testosterone (β=-1.49/quarter, 95% CI -2.71 to -0.27) were inversely associated with effusion-synovitis volume. No consistent associations were observed for men. Overall, these findings suggest endogenous sex hormones may be protective for joint structural changes in women but not men. The drop in sex hormones at the time of menopause in women may contribute to observed sex differences in knee OA.

Chapter 8 describes the independent association of magnetic resonance imaging (MRI) markers and total knee replacement (TKR) over 10.7 years in older adults aged 50 to 79

years from a general population. After adjustment for baseline pain and radiographic knee OA, cartilage defects (HR = 2.32/grade, 95% CI 1.67 to 3.21) and BMLs (HR = 2.00/grade, 95% CI 1.49 to 2.68) at baseline predicted increased risk of TKR over 10.7 years. Grade 3 total and suprapatellar effusion-synovitis (HR = 2.41, 95% CI 1.21 to 4.81 and HR = 3.96, 95% CI 1.94 to 8.09, respectively) predicted TKR over 10.7 years; however, these associations were partially dependent on other structural pathologies. Those who had TKR all had grade 3 meniscal tears at baseline. The number of pathologies was significantly associated with the risk for TKR (HR = 1.84/unit, 95% CI 1.35 to 2.51). These results demonstrate that MRI structural markers such as cartilage defects, BMLs and meniscal tears, but not effusion or meniscal extrusion in the knee were independent predictors of TKR. The risk of TKR increased as more MRI structural markers were present, suggesting that MRI structural markers are good predictors of rapid knee OA progression in the general population.

In conclusion, this series of studies indicate that knee OA is a complex disease affected by systematic factors such as low-grade inflammation, adiposity and sex hormones. Vitamin D supplementation does not significantly prevent knee cartilage loss and knee pain in patients with symptomatic knee OA. MRI structural markers are good predictors of end-stage knee OA in the general population. Recommendations for the future direction of each chapter are provided in the following section.

9.2 Future directions

OA has long been considered as a “wear and tear”disease leading to cartilage loss. Progress in molecular biology has profoundly shifted this paradigm in recent decades. Recent studies have shown that OA is a much more complex disease which involves the release of inflammatory mediators [359, 360]. Chapter 4 highlights that chronic low-grade inflammation, as reflected by increased hs-CRP, is present in patients with OA. The literature is rich in

data suggesting that inflammation play a pivotal role in the development and progression of OA. The source of inflammation could be local or systemic. While local production of inflammatory mediators are well known to contribute to cartilage degradation and synovitis, additional data show that local inflammatory events may be reflected outside the joint in peripheral blood in patients with OA. The findings of increased levels of hs-CRP in OA patients concur with the "inflammatory theory". The confirmation of low-grade inflammation in OA builds the foundation for future research to examine inflammatory factors as important components of the disease. Inflammatory biomarkers, which could readily detect sub-clinical and/or sub-acute inflammation, may be developed and included in a panel of novel diagnostic or prognostic tests for OA [361].

OA could be also initiated and/or aggravated by the presence of a systemic low-grade inflammation. The release of inflammatory mediators into the blood could be a result of aging and/or metabolic syndrome (adiposity). OA is a prototypic age-related disease, the underlying molecular mechanisms remain largely unknown. One theory is that cellular senescence could trigger internal mechanisms leading to increased production of inflammatory mediators and oxidated proteins. These mechanisms increase the concentration of reactive oxygen species (ROS) in cells, further adding to the oxidative damage triggering a low-grade inflammation [96]. The risk of OA is increased in obese patients. The increased risk cannot be explained by the mechanical effect of overload alone. Systemic factors, namely adipokines, released by abdominal adipose tissue, have been extensively studied and may contribute to the increased risk of OA in obese patients. Chapter 5 has identified significant associations between adiposity and knee pain. In particular, body fat mass is only associated with increase in non-weight-bearing knee pain, suggesting systematic mechanisms other than mechanical loading are involved. However, we could not infer the exact causal pathway underlying the relationship between adiposity and knee pain because blood samples are not collected for adipokines measurement. In future research, deciphering these inflammatory and metabolic

pathways is critical for the discovery of disease-modifying OA drugs.

Women older than 50 years have a considerably higher prevalence of OA than men of the same age group. The drop of sex hormones after menopause in women has been suggested to increase the risk of OA. The findings of Chapter 7 suggest endogenous estrogen, progesterone and testosterone are only protective for structural abnormalities in women with symptomatic knee OA but not in men. In particular, these hormones appear to be inversely associated with effusion volume, which is a clinical feature of local inflammation. Sex hormones are known to play a regulatory role in human immune response [335], therefore, the beneficial effects of sex hormones may be a result of reducing local and systemic inflammation. One randomized controlled trial showed that community dwelling women aged 50-79 years who received unopposed estrogen had modest but sustained reduction in joint pain [362] and lower rates of joint replacement [149], although this was not found in those women who received both estrogen and progesterone. However, it remains unclear whether hormone replacement therapy would prevent structural progression on MRI. Future research on the effects and potential benefits of sex hormones is needed to fully clarify their role in the development of knee OA. Also, the findings in this thesis suggest that the effects of sex hormones on MRI knee structures may differ between males and females, future studies should be sufficiently powered to conduct a gender-specific analysis to examine gender differences in the relationship between hormonal factors and the prevalence of knee OA.

Chapter 6 examines the effects of vitamin D supplementation in symptomatic knee OA patients with low vitamin D levels. Compared with placebo, vitamin D supplementation does not significantly reduce cartilage volume loss on MRI or improve WOMAC knee pain. Despite the p-values of the statistical analyses are not significant, the direction of estimated effects on all outcome measures point to favor vitamin D supplementation. In addition, modest effects are observed in secondary and post-hoc analyses on VAS knee pain, WOMAC function, 50% improvement in WOMAC pain, OMERACT-OARSI response. These findings are

largely consistent with previous two randomized clinical trials, while one showed no effects on pain and cartilage volume [129], the other one showed small effects on symptoms [130]. There are certain degree of heterogeneity among our study and the two randomized clinical trials in terms of study participants and study design, which may contribute to the inconsistencies in findings. Future research could perform a systematic review and a meta-analysis with individual patient data, which will be able to increase the power to show the true effects of vitamin D supplementation on symptoms and structural changes on MRI other than cartilage volume. A subgroup analysis according to individual patient data may be able to identify the subgroup of OA patients who would benefit the most with vitamin D supplementation.

Chapter 8 demonstrates the importance of MRI biomarkers in the prediction of TKR in the general population. The findings from our research and other studies [348] have provided evidence to support the important role of MRI in clinical decision making and practice in the context of knee OA. MRI biomarkers has the potential to provide sensitive and specific information for disease progression, to monitor treatment and help in the development of new management strategies for OA. The next step is to further to develop image analysis algorithms which may foster a more automated analysis of these MRI biomarkers and to establish a weighted scoring system which would allow standardized and overall assessment of these MRI biomarkers in clinical practice. Also, future research should focus on developing effective therapeutic interventions which target at MRI biomarkers, particularly cartilage defects and BMLs. Once disease-modifying drugs become available, quantitative MRI measures will be a particularly useful clinical tool to monitor the treatment response in large sets of OA patients.

APPENDIX A

Appendices for Chapter 4 Systematic Review

Table A.1: Results of the studies not included in the meta-analysis

Study ID	Joint	CRP measurement	No. of cases	No. of controls	Difference in hsCRP (mg/l)		
					Cases	Controls	p Value
Bos 2008	Hand	Nephelometry	353	739	1.83 (0.21–56.8)*	Not stated	0.06
Engstrom 2009	Knee	Nephelometry	89	5082	1.62	1.40	Not stated
	Hip	Nephelometry	120	5044	1.64	1.40	Not stated
Loose 1993	Not stated	ELISA	365	40	5.0 (0.1–173)*	1.9 (0.5–3.1)*	Not stated
Pearl 2007	Knee or hip	ELISA	52	Not stated	3.4 (4.7)§	0.29 (0.6) §	0.007
Punzi 2005	Hand	Nephelometry	67	31	4.7 (2.4–6.9)#	2.1 (0.5–4.9)#	0.002
Spector 1997	Knee	EIA	105	740	2.4 (1.0–5.1)#	0.7 (0.3–1.8)#	<0.001
Kerkhof 2010	Knee	Nephelometry	718	2306	1.74	1.53	0.001
	Hip	Nephelometry	349	3065	1.56	1.48	0.30
	Hand	Nephelometry	861	2164	1.56	1.48	0.12

Search Strategy for MEDLINE via Ovid (1992-2012.12)

1. Osteoarthritis/
2. Degenerative arthritis /
3. Osteoarthrosis/
4. Arthritis/
5. OR/1-4
6. C-reactive protein/
7. inflammatory marker$.tw
8. c reactive protein.tw
9. protein c reactive.tw
10. CRP.tw
11. OR/6-10
12. 5 AND 11
13. Exp Epidemiologic studies/
14. Exp Case control studies/
15. Exp Cohort studies/
16. case control.tw
17. (cohort adj (study or studies)).tw.
18. cohort analy*.tw
19. (follow up adj (study or studies)).tw.
20. (observational adj (study or studies)).tw.
21. longitudinal.tw.
22. retrospective.tw.
23. cross sectional.tw.
24. Cross-sectional studies/
25. OR/13-24
26. 12 AND 25
27. limit 26 to (English language and human)

Search Strategy for EMBASE via Ovid (1992-2012.12)

1. Osteoarthritis/
2. Degenerative arthritis /
3. Osteoarthrosis/
4. Arthritis/
5. OR/1-4
6. C-reactive protein/
7. inflammatory marker$.tw
8. c reactive protein.tw
9. protein c-reactive.tw
10. CRP.tw
11. OR/6-10
12. 5 AND 11
13. clinical study/
14. case control study/
15. family study/
16. longitudinal study/
17. retrospective study/
18. prospective study/
19. randomized controlled trials/
20. 18 NOT 19
21. cohort analysis/
22. (cohort adj (study or studies)).mp
23. (case control adj (study or studies)).tw
24. (follow up adj (study or studies)).tw.
25. (observational adj (study or studies)).tw.
26. (epidemiologic* adj (study or studies)).tw.
27. (cross sectional adj (study or studies)).tw.
28. OR/13-17, 20-27
29. 12 AND 28
30. limit 29 to (english language and human)

Search Strategy for CINAHL via EBSCO (1992-2012.12)

S1. (MH "Osteoarthritis"+)
S2. TX osteoarthritis
S3. Degenerative osteoarthritis
S4. (MH "Arthritis"+)
S5. S1 OR S2 OR S3 OR S4
S6. (MH "C-Reactive Protein")
S7. TX c reactive protein
S8. TX protein c-reactive
S9. TX crp
S10. TX inflammatory marker*
S11. S6 OR S7 OR S8 OR S9 OR S10
S12. S5 AND S11
S13. (MH "Prospective Studies"+)
S14. (MH "Case Control Studies"+)
S15. (MH "Correlational Studies")
S16. (MH "Nonconcurrent Prospective Studies")
S17. (MH "Cross Sectional Studies")
S18. S13 OR S14 OR S15 OR S16 OR S17
S19. S12 AND S18

Search for ongoing trials (Feb 10th-12th, 2013)
Key word used: 'osteoarthritis'.
Trial registry:

- ClinicalTrials.gov (http://clinicaltrials.gov/)
- WHO International Clinical Trial Registration Platform Search Portal (http://apps.who.int/trialsearch/)
- Australian and New Zealand Clinical Trial Registry (http://www.anzctr.org.au/TrialSearch.aspx)

Inclusion/Exclusion Form for Primary Studies

Study ID:
Reviewer:
Date:

Identification Details

Author	Year	Journal/ Conference	Source

On Endnote database .....Yes/ No

Full text availability ……..Yes/ No

Study Eligibility
Study design is one of the following:
Cohort study/ cross-sectional study/ case control study..... Yes/ No
The study concerns osteoarthritis..... Yes/ No
The study concerns C-reactive protein..... Yes/ No
The study is a human study, not animal/laboratory experiment…… Yes/ No

Please Tick Only One Box Below

Included	Excluded	Pending*

*** Issue relates to selective reporting – when authors may have taken CRP measurements, but not reported these within the paper. Reviewers should contact correspondent author for information on possible non-reported CRP levels & reasons for exclusion from publication. Study should be listed in 'Pending' until clarified. If no clarification is received after three attempts, study should then be excluded.**

References to Other Trials

Did this report include any references to **published reports** of potentially eligible trials not already identified for this review?		
First author	Journal / Conference	Year of publication
Did this report include any references to **unpublished data** from potentially eligible trials not already identified for this review? If yes, give list contact name and details		

Data Extraction Form

Study and Participants characteristics

Study characteristics	
	Further details
Study design (e.g. cross-section, case control*)	
Country / Countries	
Single centre / multicentre	
How was participant eligibility defined? (e.g. How osteoarthritis was defined?)	
How CRP level was measured?	
How many people were recruited?	
Duration of study (applicable to cohort, longitudinal studies)	
Other	
Participant characteristics	
	Further details
Number of participants (cases versus controls)	
Age (mean, median, range, etc)	
BMI (mean, median, range, etc)	
Female of OA participants (numbers / %, etc)	Female of OA participants (numbers / %, etc)
Type of OA (hand, knee, hip, etc)	
Other	

*** When a case control design is part of a larger clinical controlled trial, it should be regarded as case control.**

Measures relevant to the review	
Prevalence/Incidence of OA	Prevalence / Incidence/ Progression
Radiological OA	Yes / No
Clinical features	WOMAC/ Pain/ Physical Function
Others	

For dichotomous data		
OR/ RR (95% CI, p value)	Case group (n) n = number of participants, not number of events	Control group (n) n = number of participants, not number of events

Other Information (eg. adjustment, cut-off, etc):

For continuous data					
Unit	Case group		Control group		Details
	n	Mean (SD)	n	Mean (SD)	

For correlational data		
Independent factor	Correlation coefficient (r)	Adjustments

Other relevant data

Modified Newcastle – Ottawa Quality Assessment Scale

Selection:

S1. How is osteoarthritis defined?
- a) By ACR Clinical Classification Criteria for Osteoarthritis♦
- b) By Kellgren-Lawrence radiographic grade >= 2♦
- c) Total joint replacement due to primary osteoarthritis♦
- d) By record linkage (e.g. ICD codes in database) or self-reports
- e) No description

S2. Representativeness of the cases
- a) All cases over a defined period of time♦
- b) All cases in a community♦
- c) Consecutive cases from a medical setting or multicentre♦
- d) Random sample from a population♦
- e) Potential for selection biases
- f) No information on recruitment.

S3. Selection of Controls
- a) Community controls (i.e. same community as cases and would be cases if the definition of cases meets)♦
- b) Hospital controls (i.e. same community as cases but derive from a hospitalised population
- c) No description

S4. Definition of Controls
- a) No history of osteoarthritis♦
- b) No history of rheumatic joint diseases, inflammatory and malignant diseases♦
- c) No description of source

Comparability:

C1. Cases and controls are matched in age and/or age is adjusted for in the statistical analysis
- a) Yes♦
- b) No

C2. Cases and controls are matched in BMI and/or BMI is adjusted for in the statistical analysis
- a) Yes♦
- b) No

Exposure:

E1. Measurement of C-reactive protein (CRP)
- a) Description of CRP measurement♦
- b) No description

E2. Same method of measurement for cases and controls (e.g. nephelometry, ELISA)
- a) Yes♦
- b) No

E3. Non-response rate
- a) Same rate for both cases and controls♦
- b) Description of non-respondents or exclusion of data in the analysis (i.e. CRP>20 mg/l)♦
- c) Number of subjects differ in the analysis and no explanation provided

Study	S1	S2	S3	S4	C1	C2	E1	E2	E3	Total

Reviewer: _____

APPENDIX B

WOMAC Questionnaires

Menzies Research Institute Tasmania

TASOAC

ID Number | | | | |

TASOAC
Phase 4 Questionnaire

Prof. Graeme Jones

IDENTIFYING DATA

Date Phase 1 Questionnaire Completed: | | / | | / | | | | |

Date Phase 2 Questionnaire Completed: | | / | | / | | | | |

Date Phase 3 Questionnaire Completed: | | / | | / | | | | |

Date Phase 4 Questionnaire Completed: | | / | | / | | | | |

Instructions for completing the questionnaire:

All questionnaires will be submitted electronically. By following these instructions you will be assisting with this process.

Please answer all questions to the best of your ability (leave blank if unknown).
Do not put lines through irrelevant questions as it upsets the scanning machine.

Write in **BLOCK LETTERS** using the boxes where provided

Use a blue pen

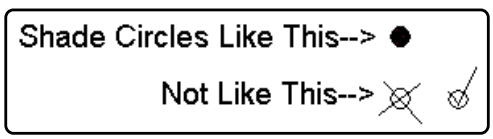
Cross out any mistake & write correct answer just below the relevant box

Indicate your response by filling in the circle next to the most appropriate answer or by writing clearly in the box or space provided.

Your answers will be completely confidential.

Please notate any queries and they will be reviewed at your appointment.

Example:



4.7 Rate the following today for your RIGHT knee

(if your **RIGHT** knee has been replaced rate your left knee)

This section assesses pain, stiffness and functional deficit on a scale from 1 - 10

Example	none									severe
Example of no pain	● 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
Example of severe pain	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	● 10

1. Referring to your knee only how much <u>pain</u> do you experience

	none									severe
a. Walking on a flat surface	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
b. Going up and down stairs	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
c. At night while in bed	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
d. Sitting or lying	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
e. Standing upright	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10

2. Referring to your knee only how much <u>stiffness</u> do you experience

	none									severe
a. After first awakening	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
b. Later in the day	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10

3. Referring to your knee only how much <u>functional deficit</u> do you experience when

	none									severe
a. Descending stairs	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
b. Ascending stairs	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
c. Rising from bed	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
d. Rising from sitting	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
e. Putting on socks	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
f. Taking off socks	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
g. Bending to the floor	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
h. Lying in bed	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10

4.7 Question 3 continued

	none									severe
i. Walking on flat surface	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
j. Getting in/out of the bath	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
k. Standing	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
l. Sitting	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
m. Getting in/out of the car	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
n. Getting on/off the toilet	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
o. Heavy domestic chores	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
p. Light domestic chores	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10
q. Shopping	○ 1	○ 2	○ 3	○ 4	○ 5	○ 6	○ 7	○ 8	○ 9	○ 10

Site Number: ☐

Randomisation Code: ☐☐☐☐

Initials: ☐☐☐

Visit Number: ☐

Date: ☐☐ / ☐☐ / ☐☐☐☐

VIDEO Study: WOMAC

Instructions: Please read carefully

Please answer all questions to the best of your ability (leave blank if unknown).

Your answers will be completely confidential.

Please place a mark on the line to rate the following today for your knee

Examples **None** **Severe**

Example of no pain |———————————

Example of severe pain ———————————|

Office use only

1. Referring to your **knees only** how much **pain** do you experience when

None **Severe**

a. Walking on a flat surface ———————————

b. Going up and down stairs ———————————

c. At night while in bed ———————————

d. Sitting or lying ———————————

e. Standing upright ———————————

2. Referring to your **knees only** how much **stiffness** do you experience

None **Severe**

a. After first awakening ———————————

b. Later in the day ———————————

3. Referring to your **knees only** how much **functional deficit** do you experience when

None **Severe**

a. Descending stairs ———————————

b. Ascending stairs ———————————

c. Rising from bed ———————————

d. Rising from sitting ———————————

e. Putting on socks ———————————

f. Taking off socks ———————————

g. Bending to the floor ———————————

h. Lying in bed ———————————

	None — Severe	*Office use only*
i. Walking on flat surface		
j. Getting in/out of the bath		
k. Standing		
l. Sitting		
m. Getting in/out of the car		
n. Getting on/off the toilet		
o. Heavy domestic chores		
p. Light domestic chores		
q. Shopping		

APPENDIX C

Published Manuscripts

These articles have been removed for copyright or proprietary reasons.

Jin, X. et al. (2015). Circulating C reactive protein in osteoarthritis: a systematic review and meta-analysis. Annals of the rheumatic diseases, 74(4), 703-10.

Jin, X. et al. (2016). Longitudinal associations between adiposity and change in knee pain: Tasmanian older adult cohort study. Seminars in arthritis and rheumatism, 45(5) 564-569

Original Investigation

Effect of Vitamin D Supplementation on Tibial Cartilage Volume and Knee Pain Among Patients With Symptomatic Knee Osteoarthritis

A Randomized Clinical Trial

Xingzhong Jin, MD; Graeme Jones, MD, PhD; Flavia Cicuttini, MD, PhD; Anita Wluka, MD, PhD; Zhaohua Zhu, MD; Weiyu Han, MD; Benny Antony, PhD; Xia Wang, MMSc; Tania Winzenberg, MD, PhD; Leigh Blizzard, PhD; Changhai Ding, MD, PhD

IMPORTANCE Observational studies suggest that vitamin D supplementation is associated with benefits for knee osteoarthritis, but current trial evidence is contradictory.

OBJECTIVE To compare the effects of vitamin D supplementation vs placebo on knee pain and knee cartilage volume in patients with symptomatic knee osteoarthritis and low vitamin D levels.

DESIGN, SETTING, AND PARTICIPANTS A multicenter randomized, double-blind, placebo-controlled clinical trial in Tasmania and Victoria, Australia. Participants with symptomatic knee osteoarthritis and low 25-hydroxyvitamin D (12.5-60 nmol/L) were enrolled from June 2010 to December 2011. The trial was completed in December 2013.

INTERVENTIONS Participants were randomly assigned to receive monthly treatment with oral vitamin D_3 (50 000 IU; n = 209) or an identical placebo (n = 204) for 2 years.

MAIN OUTCOMES AND MEASURES Primary outcomes were change in tibial cartilage volume (assessed using magnetic resonance imaging [MRI]) and change in the Western Ontario and McMaster Universities Arthritis Index (WOMAC) pain score (0 [no pain] to 500 [worst pain]) from baseline to month 24. Secondary outcomes were cartilage defects and bone marrow lesions (assessed using MRI).

RESULTS Of 413 enrolled participants (mean age, 63.2 years; 50% women), 340 (82.3%) completed the study. The level of 25-hydroxyvitamin D increased more in the vitamin D group (40.6 nmol/L) than in the placebo group (6.7 nmol/L) ($P < .001$) over 2 years. There were no significant differences in annual change of tibial cartilage volume or WOMAC pain score. There were no significant differences in change of tibiofemoral cartilage defects or change in tibiofemoral bone marrow lesions. Adverse events (≥1 per patient) occurred in 56 participants in the vitamin D group and in 37 participants in the placebo group ($P = .04$).

	Change, Mean			
End Point	Vitamin D	Placebo	Difference (95% CI)	*P* Value
Tibial cartilage volume, %/y	−3.4%	−4.2%	0.8% (−0.2% to 1.8%)	.13
WOMAC pain	−49.9	−35.1	−14.8 (−32.5 to 2.9)	.10
Tibiofemoral cartilage defects	0.3	0.5	−0.2 (−0.4 to 0.1)	.21
Tibiofemoral bone marrow lesions	−0.1	0.3	−0.5 (−0.9 to 0.0)	.06

CONCLUSIONS AND RELEVANCE Among patients with symptomatic knee osteoarthritis and low serum 25-hydroxyvitamin D levels, vitamin D supplementation, compared with placebo, did not result in significant differences in change in MRI-measured tibial cartilage volume or WOMAC knee pain score over 2 years. These findings do not support the use of vitamin D supplementation for preventing tibial cartilage loss or improving WOMAC knee pain in patients with knee osteoarthritis.

TRIAL REGISTRATION clinicaltrials.gov Identifier: NCT01176344; anzctr.org.au Identifier: ACTRN12610000495022

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Symptomatic knee osteoarthritis occurs among 10% of men and 13% of women aged 60 years or older.[1] Worldwide, knee osteoarthritis, together with hip osteoarthritis, are the 11th leading cause of global disability, accounting for 2.2% of total years lived with disability.[2] Medical costs of osteoarthritis account for 1% to 2.5% of the gross domestic product in developed countries.[3] Currently there are no disease-modifying therapies for osteoarthritis; therefore, there is a need to develop cost-effective approaches to prevent the development and progression of osteoarthritis.

Vitamin D can reduce bone turnover and cartilage degradation, thus potentially preventing the development and progression of knee osteoarthritis.[4,5] Epidemiological studies showed that low serum 25-hydroxyvitamin D levels were associated with greater knee pain,[6,7] a higher prevalence of radiographic knee osteoarthritis,[8] and higher risk of progression.[9,10] However, observational studies are subject to inherent bias and confounding factors such as physical activity and sun exposure.[11] In addition, 2 small existing randomized controlled trials (RCTs) reported contradictory results.[5,12] The inconsistencies are likely because of variations in inclusion criteria, outcome measures, follow-up time, and sample size.[13] An updated systematic review called for further larger well-designed RCTs to determine whether vitamin D supplementation can slow disease progression.[14] Therefore, we conducted an RCT of participants with clinically relevant inclusion criteria,[13] to evaluate the effects of 2 years of vitamin D supplementation vs placebo on knee pain and knee cartilage volume in patients with symptomatic knee osteoarthritis combined with low 25-hydroxyvitamin D levels. Effects on other knee structural abnormalities, including cartilage defects and bone marrow lesions, were also assessed.

Methods

Trial Design

The Vitamin D Effect on Osteoarthritis (VIDEO) study was a randomized, double-blind, placebo-controlled trial, which was conducted between June 2010 and December 2013.[15] Participants were recruited from June 2010 to December 2011 in Tasmania and Victoria, Australia, through advertisements in local media and community groups, as well as referrals from general practitioners, rheumatologists, and orthopedic surgeons. A telephone prescreening was conducted to inquire about knee pain status, comorbidities, participation in other studies, and whether the survey recipient anticipated knee or hip surgery within next 2 years. Potentially eligible participants were subsequently assessed during a clinic visit that included a physical examination, knee radiography, and assessment of serum 25-hydroxyvitamin D levels. The trial protocol appears in Supplement 1.

Participants

Inclusion and exclusion criteria were described in the published protocol.[15] In brief, eligible participants were aged 50 to 79 years, had symptomatic knee osteoarthritis (assessed according to American College of Rheumatology [ACR] criteria[16]) for at least 6 months, and had pain of 20 to 80 mm on a 100-mm visual analog scale. In addition, participants had an ACR function class rating of I, II, or III (I [complete ability to perform usual activities of daily living] to III [ability to perform usual self-care activities but limited in vocational and avocational activities]) and relatively good health score of 0 to 2 on a 5-point Likert scale (0 [very good health] to 4 [very poor health]) according to the investigator assessment of disease status. Participants were included if their serum 25-hydroxyvitamin D levels were between 12.5 nmol/L and 60 nmol/L. Ethics approval was received from the Tasmania Health and Human Medical Research Ethics Committee (reference number H1040) and Monash University Human Research Ethics Committee (reference number CF10/1182-2010000616). Informed written consent was obtained from all participants.

Exclusion criteria included grade 3 radiographic changes according to the Altman and Gold atlas,[17] severe knee pain on standing (more than 80 mm on a 100-mm visual analog scale), contraindication to magnetic resonance imaging (MRI), rheumatoid or psoriatic arthritis, lupus, cancer, severe cardiac or renal impairment, hypersensitivity to vitamin D, conditions affecting oral drug absorption, anticipated knee or hip surgery within the next 2 years, history of significant trauma of knees (eg, arthroscopy or injury to ligaments or menisci <1 year preceding the study), and history of taking vitamin D or an investigational drug within the last 30 days.

The knee that met the previously described inclusion and exclusion criteria was selected as the study knee for outcome measures. When both knees met the criteria, the study knee was defined as the one with worse pain assessed using the visual analog scale.

Randomization and Masking

Participants were allocated to either the vitamin D or placebo group at a ratio of 1:1 based on computer-generated random numbers. Allocation concealment was confirmed by a central automated allocation procedure that was independent of the investigators. Treatment assignment was masked from all participants, research coordinators, and investigators and maintained until all data were collected, confirmed for accuracy, and cleaned, and statistical analyses were performed.

Interventions

Participants in the treatment group were given a monthly capsule of 50 000 IU (1.25 mg) of vitamin D_3 (cholecalciferol) for 24 months (Nationwide Compounding Pharmacy).[18] Participants in the control group received an identical inert placebo provided by the same company.

Outcomes

Knee pain was added as an additional primary end point to the protocol on June 6, 2012, after comments were received from reviewers of the methods article.[15] The secondary outcomes included on clinicaltrials.gov slightly differ from the published protocol because several outcome measures were added in the substudies as secondary outcomes on clinicaltrial.gov, whereas the osteoarthritis outcomes were the focus in the published protocol.[15]

In this article, only the primary outcomes and a subset of secondary outcomes (as listed in the study protocol [Supplement 1]) are reported. Primary outcome measures were change in knee pain assessed using the Western Ontario and McMaster University Index of osteoarthritis (WOMAC) score[19] and change in tibial cartilage volume on MRI from baseline to month 24. There were 5 prespecified secondary outcomes (cartilage defects; tibial plateau bone area; subchondral bone marrow lesion; meniscal tear and extrusion; and lower limb muscle strength), but only the outcomes for cartilage defects and bone marrow lesions on MRI are reported in this article. Post hoc analysis outcomes include 20% and 50% improvement rates in WOMAC pain score, WOMAC function and stiffness scores, visual analog scale knee pain, and the responder criteria developed by the Outcome Measures in Rheumatology Arthritis Clinical Trials-Osteoarthritis Research Society International (OMERACT-OARSI) criteria.

Assessment of Pain

Knee pain was assessed at baseline and at months 3, 6, 12, and 24. Five items of WOMAC pain scale in 100-mm visual analog format were used to assess pain during walking, using stairs, in bed, sitting or lying, and standing. Items were summed to create a total pain score (range, 0-500).[20] Knee pain in most days of the previous month was assessed using a 100-mm visual analog scale.

Explanation of WOMAC Scoring

The total WOMAC score indicates the sum of subscale scores including pain, stiffness, and physical function. Missing responses were managed according to the WOMAC user guide.[21] The WOMAC pain score was considered void if more than 1 item was missing. In the event of a missing item, the remaining 4 items were averaged and then multiplied by 5.

OMERACT-OARSI Responder Criteria

The OARSI Standing Committee for Clinical Trials Response Criteria Initiative developed a set of responder criteria (OMERACT-OARSI) to categorize individual response to treatment as a single variable for clinical trials.[22] Response using the exact OMERACT-OARSI criteria could not be directly evaluated because patient global assessment was not recorded in this trial; therefore, we used a modified OMERACT-OARSI responder definition without patient global assessment. OMERACT-OARSI responders in this study were defined as participants with (1) at least 50% improvement and an absolute change of at least 20 points in the mean WOMAC pain score or mean WOMAC function score; or (2) at least 20% improvement and an absolute change of at least 10 points in both the mean WOMAC pain score and the mean WOMAC function score.

MRI Assessment of Knee Structural Changes

MRI scans of the study knee were obtained according to a standardized protocol using a 1.5 T whole-body MRI unit with a commercial transmit-receive extremity coil. The sequences used for cartilage volume assessment were sagittal fat saturated (FS) T1-weighted spoiled gradient echo (GRE). Cartilage defects and bone marrow lesions were assessed using T2-weighted/proton density-weighted fast spin echo (FSE) sequences. MRIs were assessed by trained readers blinded to treatment allocation according to methods described previously.[15]

Cartilage Volume | Cartilage volume was determined using the previously described image processing techniques.[8] The volumes of individual cartilage plates (medial tibial and lateral tibial) were isolated by manually drawing disarticulation contours around the cartilage boundaries on a section-by-section basis then resampled by means of bilinear and cubic interpolation for final 3-dimensional rendering using OsiriX Lite imaging software (32-bit version 5.9, Pixmeo SARL). The coefficient of variation was 2.1% for medial tibia and 2.2% for lateral tibia.[23]

Cartilage Defects | Cartilage defects (0-4) were graded on T2-weighted images using a modified Outerbridge classification[24] at medial tibial, medial femoral, lateral tibial, and lateral femoral sites (described in the protocol).[15] A total score was calculated as the total of subregional scores. Intraobserver reliability expressed as an intraclass correlation coefficient ranged from 0.77 to 0.94.

Bone Marrow Lesions | Bone marrow lesions, defined as discrete areas of increased signal adjacent to the subcortical bone, were measured using a modified Whole-Organ Magnetic Resonance Imaging Score (0 = none, 1 ≤ 25% of the subregion, 2 = 25%-50%, and 3 ≥ 50%).[25] A total score of the tibiofemoral compartment was calculated as the total of 13 subregional scores (0-39). The intraclass correlation coefficient of this bone marrow lesion ranged from 0.93 to 0.98.

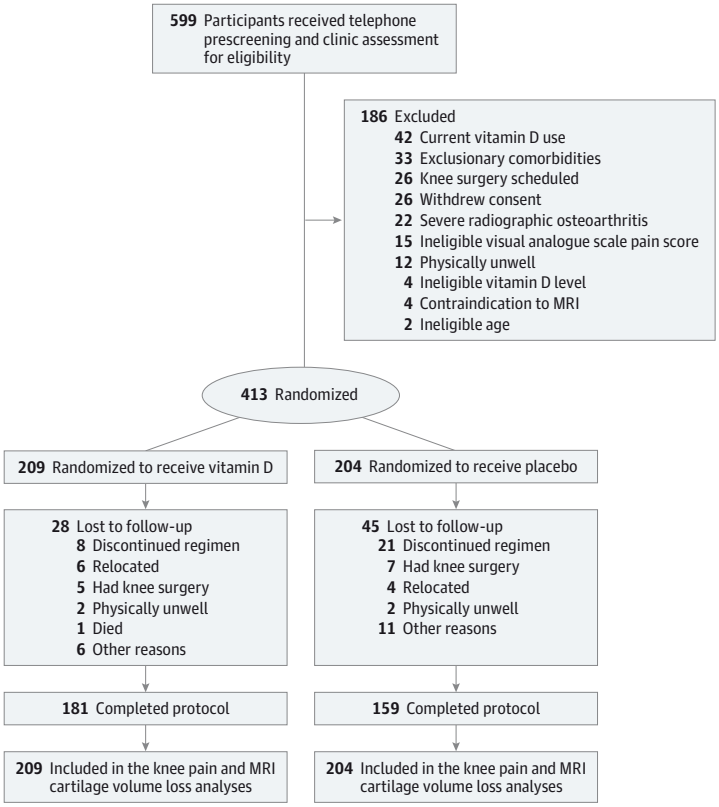
25-Hydroxyvitamin D Assays

Serum 25-hydroxyvitamin D was assayed at screening, month 3, and month 24 using direct competitive chemiluminescent immunoassays (DiaSorin Inc). The intraassay and interassay coefficients of variation were 3.2% and 6.0%.

Sample Size

Quantification of cartilage volume loss has been used to monitor the progression of knee osteoarthritis.[26] Previous studies reported that mean annual loss of medial tibial cartilage volume loss in patients with knee osteoarthritis was 4.5%.[27] Monthly intake of 50 000 IU of vitamin D would achieve serum 25-hydroxyvitamin D levels greater than 60 nmol/L[28] and this change was estimated to lead to an absolute reduction in medial tibial cartilage loss of 2.2% annually,[8] which was expected to translate into a risk reduction of 44% for total knee replacement over 4 years.[29] Sample size calculation assumed $\alpha = .05$ and $\beta = .20$ and was performed based on the Cohen formula.[30] We calculated that 400 participants at baseline (200 in each group), allowing 20% for dropouts, would have at least 80% power to detect a 2.2% between-group difference in medial tibial cartilage loss. For change in WOMAC pain, we anticipated a standard deviation of 70.5 on a score from 0 to 500.[12] With 400 partici-

Figure 1. Flowchart of Participants in the VIDEO Study



MRI indicates magnetic resonance imaging.

pants, a difference between groups of 20 units on the score is detectable with 80% power.

Statistical Analysis

WOMAC and visual analog scale knee pain scores were analyzed using a repeated-measures mixed model with terms for age, sex, body mass index (calculated as weight in kilograms divided by height in meters squared), treatment, month, and trial center. The correlation within the repeated measures was addressed by using an individual participant identification as a random effect. The effect of treatment was evaluated by the month × treatment interaction. In a post hoc analysis, the proportion of participants who achieved at least 20% and 50% improvement in WOMAC pain score was evaluated, which has been shown to be clinically relevant.[31]

The independent *t* test was used to compare annual changes in cartilage volume and absolute changes of cartilage defects and bone marrow lesions between groups. An increase in cartilage defects and bone marrow lesions was defined as a change of more than 1 unit in score. Presence of an increase in cartilage defects or bone marrow lesions was compared between the 2 groups using logistic regression.

Both intention-to-treat and per-protocol analyses were used. Per-protocol analysis was defined as achieving a 25-hydroxyvitamin D level of greater than 60 nmol/L at the month 3 visit. Multiple imputation by chained equations was used to address missing data caused by loss to follow-up and nonresponses. Imputations were performed separately for each treatment group and each outcome using baseline values, age, sex, body mass index, and serum 25-hydroxyvitamin D level. All statistical analyses were performed using Stata version 13.0 (Stata Corporation) and a 2-sided *P* value of .05 was considered statistically significant.

Results

Participants

Figure 1 shows the flow of study participants. A total of 599 participants were screened for eligibility from June 5, 2010, to December 1, 2011, and 413 participants were randomly assigned to receive either vitamin D (n = 209) or placebo (n = 204). The mean age of participants was 63.2 years, 208 (50%) were women, and mean body mass index was 29.6. Participants' demographic characteristics were comparable at baseline between 2 groups (**Table 1**). Seventy-three participants withdrew from the study (28 [13.4%] in the vitamin D group and 45 [22.1%] in the placebo group [*P* = .02]) and 340 participants (82.3%) completed the trial. There were no significant differences between participants who completed the study vs those who did not, except that among those who withdrew, more were women and had lower tibial cartilage volume (eTable in Supplement 2). Fewer participants discontinued treatment in the vitamin D group (8) than the placebo

group (21). The major reason for a higher drop-out rate in the placebo group was that participants had their 25-hydroxyvitamin D levels checked by a primary care physician and started taking vitamin D after finding low vitamin D levels. Consequently, they were withdrawn from the study. All available data from the randomized participants were included in the intention-to-treat analyses.

Primary End Points

The mean serum 25-hydroxyvitamin D level increased by 40.6 nmol/L in vitamin D group and by 6.7 nmol/L in placebo group over 2 years. Overall, 165 (79%) participants in the vitamin D group and 88 (43%) participants in the placebo group reached a 25-hydroxyvitamin D level of greater than 60 nmol/L at month 3.

Changes in WOMAC knee pain are presented in **Table 2**. At baseline, the mean (SD) of WOMAC pain scores were 137.9 (88.8) in the vitamin D group and 134.7 (83.4) in the placebo group (difference, 3.2 [95% CI, −13.5 to 19.8]; P = .71). Total WOMAC pain decreased over 24 months in both groups (**Figure 2**A). At month 24, the mean (SD) of WOMAC pain scores were 87.0 (90.1) in the vitamin D group and 97.2 (87.5) in the placebo group (difference, −10.2 [95% CI, −28.8 to 8.4]; P = .28). There was no difference in change in WOMAC pain between groups in the mixed-effect model in which all time points were included (−49.9 for the vitamin D group vs −35.1 for the placebo group; between-group difference, −14.8 [95% CI, −32.5 to 2.9]; P = .10).

Tibial cartilage volume (mean [SD]) at baseline was not different between the vitamin D group (3466 mm^3 [1038]) and the placebo group (3640 mm^3 [1036]) (between-group difference, −174 mm^3 [95% CI of difference, −375 to 27]; P = .09). At month 24, tibial cartilage volume was also not different between the vitamin D group (3238 mm^3 [989]) and the placebo group (3398 mm^3 [1030]) (between-group difference, −160 mm^3 [95% CI of difference, −369 to 49]; P = .13). Change in tibial cartilage volume (Table 2) was not different between the groups (−242.6 mm^3 for the vitamin D group vs −301.4 mm^3 for the placebo group [between-group difference, 58.8 mm^3 {95% CI, −13.9 to 131.4}]; P = .11). Per-protocol analysis comparing participants who achieved a 25-hydroxyvitamin D level of greater than 60 nmol/L at their month 3 visit (n = 253) with those who did not (n = 146) (14 participants withdrew within 3 months) showed similar results (−261.9 mm^3 vs −284.8 mm^3 [between-group difference, 22.9 mm^3 {95% CI, −51.5 to 97.3}]; P = .55).

Secondary End Points

The results for tibiofemoral cartilage defects and bone marrow lesions are shown in Table 2. The difference in cartilage defect score was not different between groups. Bone marrow lesion scores decreased in both groups and no significant difference was observed.

Post Hoc Analyses

In post hoc analyses (Table 2), participants in the vitamin D group had statistically significant improvements in visual analog scale knee pain (Figure 2E) scores when compared with the placebo group. The vitamin D group had more improvement in the total WOMAC score (Figure 2B) and WOMAC function (Figure 2C) but not WOMAC stiffness (Figure 2D). There were 115 (64%) participants in the vitamin D group and 95 (57%) participants in the placebo group (P = .16) who achieved a 20% improvement in WOMAC knee pain score over 2 years. There were 90 (50%) participants in the vitamin D group and 65 (39%) participants in the placebo group (P = .04) who showed at least a 50% improvement in WOMAC pain score. There were more OMERACT-OARSI responders in the vitamin D group (74/209 [35%]) than the placebo group (52/204 [25%]) (P = .03). The proportion of participants who had an increase in bone marrow lesions was lower in vitamin D group (44/183 [24%]) than in the placebo group (61/175 [35%]) (P = .03).

Table 1. Baseline Characteristics of the Vitamin D and the Placebo Groups

	Vitamin D (n = 209)	Placebo (n = 204)
Study site		
Hobart, No. (%)	129 (61)	132 (64)
Melbourne, No. (%)	80 (38)	72 (35)
Age, mean (SD), y	63.5 (6.9)	62.9 (7.2)
Women, No. (%)	106 (50)	102 (50)
Body mass index, mean (SD)[a]	29.6 (5.4)	29.6 (4.6)
Serum 25-hydroxyvitamin D, mean (SD), nmol/L	43.7 (11.8)	43.8 (12.7)
Radiographic osteoarthritis, No. (%)	163 (96)	157 (96)
Total WOMAC score (0-2400), mean (SD)[b]	687.3 (426.3)	664.7 (390.8)
Pain (0-500)	137.9 (88.8)	134.7 (83.4)
Stiffness (0-200)	61.5 (41.5)	61.7 (40.1)
Function (0-1700)	487.9 (318.1)	467.6 (292.8)
Visual analog scale pain (0-100 mm), mean (SD)[b]	48.7 (21.4)	46.4 (20.5)
Tibial cartilage volume, mean (SD), mm^3	3466 (1038)	3640 (1036)
Medial tibial region	1461 (463)	1522 (474)
Lateral tibial region	2005 (716)	2118 (681)
Cartilage defects, No. (%)	194 (93)	192 (94)
Medial tibiofemoral region	176 (84)	164 (80)
Lateral tibiofemoral region	159 (76)	157 (77)
Bone marrow lesions, No. (%)	134 (64)	147 (72)
Medial tibiofemoral region	90 (43)	95 (46)
Lateral tibiofemoral region	81 (39)	89 (43)

Abbreviation: WOMAC, Western Ontario and McMaster University Index.

[a] Body mass index was calculated as weight in kilograms divided by height in meters squared.

[b] Higher scores in WOMAC and the visual analog scale pain indicate a more severe stage of the condition.

Adverse Events

Fifty six of the 209 participants (27%) in the vitamin D group reported at least 1 adverse event vs 37 of the 204 participants (18%) in the placebo group (**Table 3**). Four participants developed hypercalcemia in the vitamin D group vs 2 in the placebo group. One participant in the vitamin D group had symptoms of hyperparathyroidism (eg, muscle cramps,

Table 2. Change in Study End Points Over 2 Years Between Vitamin D and Placebo Groups, Mean (95% CI)[a]

	Vitamin D (n = 209)			Placebo (n = 204)				
	Mean (SD)			Mean (SD)				
	Baseline	Month 24	Change, Mean (95% CI)[b]	Baseline	Month 24	Change, Mean (95% CI)[b]	Between-Group Difference in Change, Mean (95% CI)[b,c]	*P* Value
Primary End Points								
WOMAC pain (0-500)	137.9 (88.8)	87.0 (90.1)	−49.9 (−62.2 to −37.6)	134.7 (83.4)	97.2 (87.5)	−35.1 (−47.8 to −22.4)	−14.8 (−32.5 to 2.9)	.10
Tibial cartilage volume, mm^3	3466 (1038)	3238 (989)	−242.6 (−294.6 to −190.6)	3640 (1036)	3398 (1030)	−301.4 (−254.7 to −248.0)	58.8 (−13.9 to 131.4)	.11
Medial tibial cartilage volume, mm^3	1461 (463)	1369 (464)	−105.0 (−134.8 to −75.3)	1522 (474)	1407 (456)	−136.7 (−167.3 to −106.1)	31.7 (−10.0 to 73.4)	.14
Lateral tibial cartilage volume, mm^3	2005 (716)	1870 (673)	−138.0 (−171.2 to −104.8)	2118 (681)	1992 (689)	−164.7 (−198.5 to −130.9)	26.7 (−19.4 to 72.9)	.26
Change in tibial cartilage volume (%/y)			−3.4 (−4.2 to −2.7)			−4.2 (−5.0 to −3.5)	0.8 (−0.2 to 1.8)	.13
Medial tibial cartilage volume (%/y)			−3.3 (−4.3 to −2.3)			−4.4 (−5.4 to −3.4)	1.1 (−0.3 to 2.5)	.12
Lateral tibial cartilage volume (%/y)			−3.4 (−4.3 to −2.6)			−4.1 (−5.0 to −3.2)	0.7 (−0.6 to 1.8)	.29
Secondary End Points								
Tibiofemoral cartilage defects (0-12)	9.3 (3.1)	9.7 (3.2)	0.3 (0.1 to 0.5)	8.7 (2.9)	9.2 (3.1)	0.5 (0.3 to 0.6)	−0.2 (−0.4 to 0.1)	.21
Medial tibiofemoral defects (0-6)	4.9 (2.1)	5.2 (2.1)	0.2 (0.1 to 0.3)	4.6 (2.1)	5.0 (2.2)	0.3 (0.2 to 0.5)	−0.1 (−0.3 to 0.1)	.20
Lateral tibiofemoral defects (0-6)	4.4 (1.9)	4.5 (1.9)	0.1 (−0.1 to 0.2)	4.1 (1.8)	4.2 (1.9)	0.1 (0.0 to 0.3)	−0.1 (−0.2 to 0.1)	.57
Tibiofemoral bone marrow lesions (0-39)	2.4 (2.9)	2.3 (3.0)	−0.1 (−0.4 to 0.2)	2.6 (2.9)	2.9 (3.7)	0.3 (0.00 to 0.7)	−0.5 (−0.9 to 0.0)	.06
Medial tibiofemoral lesions (0-18)	1.3 (2.2)	1.2 (2.1)	−0.1 (−0.3 to 0.1)	1.4 (2.3)	1.5 (2.6)	0.1 (−0.1 to 0.4)	−0.2 (−0.5 to 0.1)	.19
Lateral tibiofemoral lesions (0-18)	0.8 (1.4)	0.8 (1.4)	−0.1 (−0.2 to 0.1)	0.9 (1.3)	1.0 (1.6)	0.2 (0.0 to 0.3)	−0.2 (−0.5 to 0.0)	.09
Post hoc End Points								
WOMAC total (0-2400)	687.3 (426.3)	434.3 (419.3)	−239.2 (−290.5 to −188.0)	664.7 (390.8)	504.7 (435.7)	−147.8 (−200.8 to −94.9)	−91.4 (−165.1 to −17.7)	.02
Function (0-1700)	487.9 (318.1)	306.4 (303.7)	−170.2 (−207.4 to −133.0)	467.6 (292.8)	361.8 (322.8)	−97.3 (−135.7 to −58.8)	−72.9 (−126.4 to −19.4)	.008
Stiffness (0-200)	61.5 (41.5)	41.1 (44.1)	−19.7 (−25.4 to −13.9)	61.7 (40.1)	45.7 (41.1)	−15.4 (−21.3 to −9.5)	−4.2 (−12.5 to 4.0)	.31
Visual analog scale pain (0-100)	48.7 (21.4)	33.7 (27.1)	−14.8 (−18.5 to −11.1)	46.4 (20.5)	36.4 (25.1)	−9.4 (−13.3 to −5.6)	−5.4 (−10.7 to −0.1)	.05

Abbreviations: WOMAC, Western Ontario and McMaster University Index.

[a] Higher scores in WOMAC, visual analog scale pain, cartilage defects, and bone marrow lesions indicate a more severe stage of the condition.

[b] Change in WOMAC scores and visual analog scale pain results are generated from mixed models adjusted for age, sex, body mass index, center, and month. Change in cartilage volume, cartilage defect, and bone marrow lesion results are generated from multiple imputed data sets.

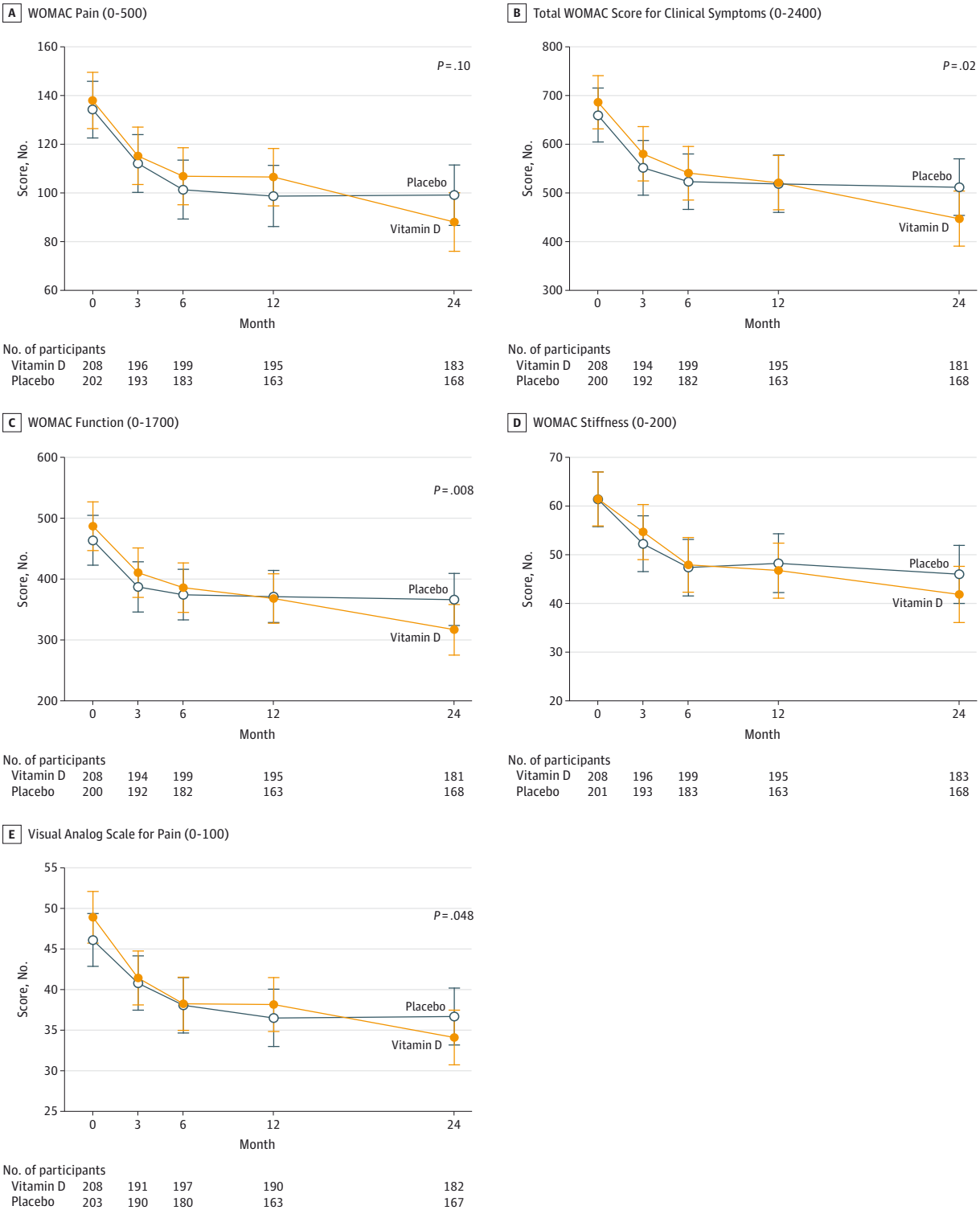
[c] Between-group differences were calculated using vitamin D group values minus placebo group values.

brittle bones, and kidney dysfunction) vs none in the placebo group. There was 1 episode of renal calculus in each group.

Discussion

The purpose of this RCT was to determine whether vitamin D supplementation could reduce knee pain and cartilage loss and also prevent progression of other knee structural abnormalities in patients with knee osteoarthritis and low 25-hydroxyvitamin D levels. Results showed that even among study participants with low 25-hydroxyvitamin D, supplementation did not slow cartilage loss or improve WOMAC-assessed pain. These data suggest a lack of evidence to support vitamin D supplementation for slowing disease progression or structural change in knee osteoarthritis.

Figure 2. Comparison Between Vitamin D Group and Placebo Group on Change in Clinical Symptoms: the WOMAC Pain Assessment and the Visual Analog Scale for Pain



Higher scores for the Western Ontario and McMaster University Index (WOMAC) and the visual analog scale indicate more severe symptoms of disease. Vertical bars indicate 95% CIs for the mean scores. *P* values indicate statistical significance between the 2 groups in score change from baseline to month 24.

Table 3. Adverse Events

	No. (%) of Participants	
	Vitamin D (n = 209)	Placebo (n = 204)
Serious adverse events		
Death	1 (0.5)	0
Malignancy	4 (1.9)	2 (1.0)
Coronary artery disease	1 (0.5)	1 (0.5)
Severe infection	0	3 (1.5)
Major depression	1 (0.5)	0
Nephrolithiasis	1 (0.5)	1 (0.5)
Hospitalization[a]	3 (1.4)	0
Adverse events		
Hypercalcemia	4 (1.9)	2 (1.0)
Hyperparathyroidism	1 (0.5)	0
Renal	2 (1.0)	0
Falls	2 (1.0)	0
Musculoskeletal	1 (0.5)	1 (0.5)
Neurological	5 (2.4)	4 (2.0)
Gastrointestinal	7 (3.3)	5 (2.5)
Respiratory	2 (1.0)	2 (1.0)
Ocular	1 (0.5)	2 (1.0)
Infection	6 (2.9)	4 (2.0)
Cardiac arrhythmia	3 (1.4)	0
Chest pain	4 (1.9)	5 (2.5)
Pain	7 (3.3)	2 (1.0)
Allergy/immunology	0	2 (1.0)
Other events[b]	9 (4.3)	7 (3.4)

[a] Two participants were admitted to the hospital after a fall and 1 was admitted because of severe diarrhea.

[b] Includes headache, lethargy, flu symptoms, and other events (neuroma, dysphonia, hypotension, lipoma, hypersensitivity, and Sjögren syndrome).

Although epidemiological studies suggest that knee osteoarthritis is more prevalent among individuals who are deficient in vitamin D, and vitamin D deficiency is associated with cartilage loss[8-10] and knee osteoarthritis symptoms,[6,7,32] the results from 2 prior RCTs were mixed. In one study, supplementation of vitamin D_3 (2000 IU/day) over 2 years showed no benefit for symptoms and structural changes in patients with knee osteoarthritis, regardless of their 25-hydroxyvitamin D levels.[5] The other study reported a small but statistically significant benefit on symptoms in patients with vitamin D insufficiency over 1 year.[12] Both studies have limitations. The first study included patients without vitamin D deficiency who may not benefit from vitamin D supplementation and patients whose disease was too severe to respond to vitamin D treatment. Also, it had a small sample size (146 participants).[13] The second study did not examine structural changes and had a 1-year follow-up, which may be too short to observe disease progression.[33] Our study addressed these limitations by recruiting patients without severe knee osteoarthritis with low 25-hydroxyvitamin D levels and provided follow-up for 2 years. Nonetheless, our results are largely consistent with the prior 2 trials.

Structural changes in cartilage and noncartilaginous joint tissue assessed using MRI, are now recommended outcomes for clinical trials in osteoarthritis.[34] An observational study showed that lower serum 25-hydroxyvitamin D levels were associated with greater cartilage volume loss over 2.7 years.[8] In the current study, the amount of tibial cartilage volume loss in the placebo group is consistent with the findings of a previous RCT.[5] We did not find significant effects on change in knee cartilage defects and bone marrow lesions.

Adverse effects of vitamin D use may include hypercalcemia. Although intermittent use of very high-dose vitamin D (eg, 500 000 IU/year) may not be safe,[35] our study suggests that a monthly regimen at a dosage of 50 000 IU is safe in elderly patients, even though the serum 25-hydroxyvitamin D levels of long-term users are at the upper limit of the normal range.[36]

The key strength of this RCT is the inclusion and exclusion criteria. This study included only adult patients with knee osteoarthritis who had a vitamin D insufficiency—patients who may be the most likely to benefit from vitamin D supplements. We also used a predefined range of knee pain to prevent a ceiling or floor effect in the statistical analyses. Patients with late-stage knee osteoarthritis were excluded because of very little cartilage remaining; thus, any possible benefits of therapy on cartilage would be difficult to identify. By using these criteria, we studied a patient population in whom the likelihood of demonstrating an effect (if truly present) of vitamin D supplementation was maximized.

This study also had limitations. First, WOMAC pain as a second primary outcome was added during the recruitment period at the time the protocol was published. However, this change was made before the trial was completed, before any data analyses, and the original sample size had sufficient power to detect the expected difference in WOMAC pain. Second, loss to follow-up was 17.7% and was less in the vitamin D group (28 participants) than in the placebo group (45 participants) (P = .02). There were fewer participants who withdrew from their assigned intervention in the vitamin D group (n = 8) than in the placebo group (n = 21). Participants who did not adhere to their assigned intervention could be expected to have a worse outcome than those who did. Although this could bias the result toward the null, similar results were seen in per-protocol analysis, suggesting the differential dropout rate had minimal effects on our results. Last, this study did not prespecify clinical outcomes such as visual analog scale knee pain and WOMAC physical function as primary or secondary end points.

Conclusions

Among patients with symptomatic knee osteoarthritis and low serum 25-hydroxyvitamin D levels, vitamin D supplementation, when compared with placebo, did not result in significant differences in change in MRI-measured tibial cartilage volume or change in WOMAC knee pain score over 2 years. These findings do not support the use of vitamin D supplementation for preventing tibial cartilage loss or improving WOMAC knee pain among patients with knee osteoarthritis.

Appendix C. Published Manuscripts

ARTICLE INFORMATION

Author Contributions: Dr Ding had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs Ding, Jin, and Jones contributed equally to this study.
Study concept and design: Ding, Jones, Cicuttini, Winzenberg.
Acquisition, analysis, or interpretation of data: All authors.
Drafting of the manuscript: Jin, Cicuttini, Zhu, Antony, Winzenberg, Blizzard, Ding.
Critical revision of the manuscript for important intellectual content: All authors.
Statistical analysis: Jin, Zhu, Antony, Wang, Blizzard, Ding.
Obtained funding: Ding, Jones, Cicuttini, Wluka, Winzenberg.
Administrative, technical, or material support: All authors.
Study supervision: Ding, Jones, Cicuttini.

Conflict of Interest Disclosures: All authors have completed and submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Dr Jones reports being the recipient of a National Health and Medical Research Council (NHMRC) Practitioner Fellowship. Drs Wluka and Winzenberg report being recipients of NHMRC Career Development Fellowship. Dr Ding reports being the recipient of an Australian Research Council Future Fellowship. No other disclosures were reported.

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