NOVEL AND SYSTEMIC RISK FACTORS FOR KNEE AND HIP OSTEOARTHRITIS

Sultana Monira Hussain

Bachelor of Medicine, Bachelor of Surgery

Master of Public Health

A thesis submitted in fulfillment of the requirement for the degree of

Doctor of Philosophy

Department of Epidemiology and Preventive Medicine

School of Public Health and Preventive Medicine

Faculty of Medicine, Nursing and Health Sciences

Monash University

December 2014

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Abstract

Osteoarthritis (OA) is a major public health problem and the most common cause of disability. Growing evidence suggests that OA is a disease of the whole joint. However, the etiology and risk factors of OA have not been fully elucidated, and there is no registered disease modifying drug to halt disease progression. Over recent years, new approaches and initiatives have been adapted for better understanding the disease pathology and mechanisms underlying the risk factors, such as the role of hormonal factors, metabolic and vascular factors, vitamin D and birth weight. This project attempted to investigate these systemic and novel risk factors for the incidence of knee and hip replacement for OA in linkage studies.

In this thesis knee and hip replacement for OA were used as a surrogate for severe OA which provides evidence of the true problem and signifies the economic burden. The study populations were the participants of the Melbourne Collaborative Cohort Study and the Australian Diabetes, Obesity and Lifestyle Study which were linked to the Australian Orthopaedic Association National Joint Replacement Registry to determine the incidence of knee and hip replacement for OA.

The initial focus of this thesis was on the association between hormonal factors and risk of total knee and hip replacement for OA. A lower estradiol concentration was a risk factor for total knee replacement for OA, while a lower androstenedione concentration and higher Sex Hormone Binding Globulin concentration were risk factors for total hip replacement for OA in women. Moreover, a lower index-to-ring finger length ratio, a proxy indicator of in-utero testosterone exposure, was associated with an increased risk of total knee replacement for OA but not the risk of total hip replacement for OA.

The second part of this thesis examined the relationship between metabolic and vascular factors and the risk of knee and hip replacement for OA. The metabolic syndrome and cumulative number of metabolic syndrome components were both associated with increased risk of total knee replacement for OA, with no association observed for total hip replacement for OA. Additionally, retinal arteriolar narrowing was associated with increased risk of knee replacement for OA. These findings suggest that metabolic and vascular factors play a role in the pathogenesis of knee OA.

The third part of this thesis examined the association between serum 25-hydroxy-vitamin D concentrations and the risk of hip replacement for OA. Higher serum 25-hydroxy-vitamin D concentrations were associated with an increased risk of hip replacement for OA in males but not in females.

Finally, this thesis examined the association between birth weight and risk of knee and hip replacement for OA. Individuals born with low birth weight or preterm birth were at increased risk of hip replacement for OA but not knee replacement for OA in adult life.

In conclusion, this thesis has made major contribution to the understanding of the role of hormonal factors, metabolic and vascular factors, serum 25-hydroxy-vitamin D concentration and birth weight in the pathogenesis of knee and hip OA. This thesis has highlighted the significant heterogeneity in terms of the risk factors for OA and that knee and hip OA are susceptible to different risk factors. This study has identified novel targets for the prevention and treatment of knee and hip OA separately that are likely to have an impact on this debilitating disease.

Declaration

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

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

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Date:

Monash University

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

General Declaration

In accordance with Monash University Doctorate Regulation 17.2 Doctor of Philosophy and Research Master's regulations the following declarations are made:

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

This thesis includes 4 original papers published in peer reviewed journals and 2 unpublished manuscripts. The core theme of the thesis is novel and systemic risk factors for knee and hip osteoarthritis. The ideas, development and writing up of all the papers in the thesis were the principal responsibility of myself, the candidate, working within the School of Public Health and Preventive Medicine under the supervision of Professor Flavia Cicuttini and Dr Yuanyuan Wang.

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

In the case of chapters 3 to 6 my contribution to the work involved the following:

Thesis chapter	**Publication title**	**Publication status**	**Nature and extent of candidate's contribution**
3	Incidence of total knee and hip replacement due to osteoarthritis in relation to circulating sex steroid hormone concentrations in women	Published	Data analysis and interpretation, manuscript development and preparation
3	Association between index-to-ring finger length ratio and risk of severe knee and hip osteoarthritis requiring total joint replacement	Published	Data analysis and interpretation, manuscript development and preparation
4	Incidence of total knee and hip replacement for osteoarthritis in relation to the metabolic syndrome and its components: A prospective cohort study	Published	Data analysis and interpretation, manuscript development and preparation
4	Retinal arteriolar narrowing and incidence of knee replacement for osteoarthritis: a prospective cohort study	Under revision	Study design, data analysis and interpretation, manuscript development and preparation
5	Is serum level of 25-hydroxyvitamin D associated with the risk of hip replacement	Under review	Study design, data analysis and interpretation, manuscript development

Thesis chapter	Publication title	Publication status	Nature and extent of candidate's contribution
	for osteoarthritis? Results from a prospective cohort study		and preparation
6	Association of low birth weight and preterm birth with the incidence of knee and hip arthroplasty for osteoarthritis	In press	Study design, data analysis and interpretation, manuscript development and preparation

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

Signed:

Date:

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Publications and awards

Publications included in this thesis

1. **Hussain SM**, Cicuttini FM, Bell RJ, Robinson PJ, Davis SR, Giles GG, Graves S, Milne RL, Wang Y. Incidence of total knee and hip replacement due to osteoarthritis in relation to circulating sex steroid hormone concentrations in women. Arthritis & Rheumatology 2014;66(8):2144-51. (IF 7.871)

2. **Hussain SM,** Wang Y, Muller DC, Wluka AE, Giles GG, Manning JT, Graves S, Cicuttini FM. Association between index-to-ring finger length ratio and risk of severe knee and hip osteoarthritis requiring total joint replacement. Rheumatology (Oxford) 2014;53(7):1200-7. (IF 4.435)

3. **Hussain SM,** Wang Y, Cicuttini FM, Simpson JA, Giles GG, Graves S, Wluka AE. Incidence of total knee and hip replacement for osteoarthritis in relation to the metabolic syndrome and its components: A prospective cohort study. Semin Arthritis Rheum 2014;43(4):429-36. (IF 3.629)

4. **Hussain SM,** Wang Y, Shaw JE, Magliano DJ, Wong TY, Wluka AE, Graves S, Tapp RJ, Cicuttini FM. Retinal arteriolar narrowing and incidence of knee replacement for osteoarthritis: a prospective cohort study. Osteoarthritis & Cartilage. (Under Revision)

5. **Hussain SM,** Daly RM, Wang Y, Shaw JE, Magliano DJ, Graves S, Ebeling PR, Wluka AE, Cicuttini FM. Is serum level of 25-hydroxyvitamin D associated with the

risk of hip replacement for osteoarthritis? Results from a prospective cohort study. (Under Review)

6. **Hussain SM,** Wang Y, Wluka AE, Shaw JE, Magliano DJ, Graves S, Cicuttini FM. Association of low birth weight and preterm birth with the incidence of knee and hip arthroplasty for osteoarthritis. Arthritis Care & Research. 2014 Nov 3. doi: 10.1002/acr.22475. [Epub ahead of print] (IF 4.039)

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Conference presentations during candidature

1. **Hussain SM**, Wang Y, Wluka AE, Shaw J, Magliano D, Graves S, Cicuttini FM. Association of low birth weight and preterm birth with the incidence of knee and hip arthroplasty for osteoarthritis. American College of Rheumatology Annual Meeting, Boston, November 2014. Poster presentation

2. **Hussain SM**, Wang Y, Shaw J, Magliano D, Wong TY, Wluka AE, Graves S, Tapp RJ, Cicuttini FM. Retinal arteriolar narrowing and incidence of knee replacement for osteoarthritis: the Australian Diabetes, Obesity and Lifestyle (AusDiab) Study. American College of Rheumatology Annual Meeting, Boston, November 2014. Poster presentation

3. **Hussain SM**, Wang Y, Wluka AE, Shaw J, Magliano D, Graves S, Cicuttini FM. Association of low birth weight and preterm birth with the incidence of knee and hip arthroplasty for osteoarthritis. AMREP ECR conference, Melbourne, September 2014. Poster presentation

4. **Hussain SM**, Wang Y, Wluka AE, Shaw J, Magliano D, Graves S, Cicuttini FM. Association of low birth weight and preterm birth with the incidence of knee and hip arthroplasty for osteoarthritis. American College of Rheumatology Annual Meeting, Boston, August 2014. Poster presentation

5. **Hussain SM**, Wang Y, Wluka AE, Shaw J, Magliano D, Graves S, Cicuttini FM. Association of low birth weight and preterm birth with the incidence of knee and hip

arthroplasty for osteoarthritis. Australian Rheumatology Association, Victoria, November 2014. Oral presentation

6. **Hussain SM**, Wang Y, Wluka AE, Shaw J, Magliano D, Graves S, Cicuttini FM. Association of low birth weight and preterm birth with the incidence of knee and hip arthroplasty for osteoarthritis. Australian Rheumatology Association, Annual Scientific Meeting, Hobart, May 2014. Poster presentation

7. **Hussain SM**, Cicuttini FM, Bell RJ, Robinson PJ, Davis SR, Giles GG, Graves S, Wang Y. Incidence of total knee and hip replacement due to osteoarthritis in relation to circulating sex steroid hormone concentrations in women. Australian Rheumatology Association, Annual Scientific Meeting, Hobart, May 2014. Poster presentation

8. **Hussain SM**, Wang Y, Shaw J, Magliano D, Wong TY, Wluka AE, Graves S, Tapp RJ, Cicuttini FM. Retinal arteriolar narrowing and incidence of knee replacement for osteoarthritis: the Australian Diabetes, Obesity and Lifestyle (AusDiab) Study. Australian Rheumatology Association, Annual Scientific Meeting, Hobart, May 2014. Poster presentation

9. **Hussain SM**, Cicuttini FM, Bell RJ, Robinson PJ, Davis SR, Giles GG, Graves S, Wang Y. Incidence of total knee and hip replacement due to osteoarthritis in relation to circulating sex steroid hormone concentrations in women. World Congress on Osteoarthritis. Paris, France. April 2014. Poster presentation

10. **Hussain SM**, Wang Y, Wluka AE, Shaw J, Magliano D, Graves S, Cicuttini FM. Association of low birth weight and preterm birth with the incidence of knee and hip arthroplasty for osteoarthritis. World Congress on Osteoarthritis. Paris, France. April 2014. Poster presentation

11. **Hussain SM**, Wang Y, Cicuttini FM, Simpson JA, Giles GG, Graves SE, Wluka AE. Incidence of severe knee and hip osteoarthritis in relation to the metabolic syndrome and its components: a prospective cohort study. Australia and New Zealand Obesity Society, Annual Scientific Meeting, Melbourne, October 2013. Poster presentation

12. **Hussain SM**, Wang Y, Cicuttini FM, Simpson JA, Giles GG, Graves SE, Wluka AE. Incidence of severe knee and hip osteoarthritis in relation to the metabolic syndrome and its components: a prospective cohort study. European League Against Rheumatism, EULAR, Annual European Congress of Rheumatology, Madrid, Spain, June 2013. Oral presentation

List of abbreviations

25(OH)D	Serum 25-hydroxy-vitamin D
2D:4D	The ratio of the length of the index (2D) and ring (4D) fingers
ACR	American College of Rheumatology
AOA NJRR	Australian Orthopaedic Association National Joint Replacement Registry
ASD	Androstenedione
AusDiab	Australian Diabetes, Obesity and Lifestyle Study
BMI	Body mass index
BML	Bone marrow lesion
BPH	Benign prostatic hyperplasia
COPD	Chronic obstructive pulmonary disease
DDH	Developmental dysplasia of the hip
DHEAS	Dehydroepiandrosterone sulfate
E2	Estradiol
EULAR	European League Against Rheumatism
GBD	Global Burden of Disease
HR	Hazard ratio
HRT	Hormone replacement therapy
LBW	Low birth weight
MCCS	Melbourne Collaborative Cohort Study
MetS	Metabolic syndrome
OA	Osteoarthritis
ROAD	Research on Osteoarthritis / Osteoporosis Against Disability

SD	Standard deviation
SHBG	Sex hormone binding globulin
T	Testosterone
TNF α	Tumour necrosis factor α
YLD	Years lived with disability

Chapter 1: General introduction

1.1 Overview of osteoarthritis

Osteoarthritis (OA), one of the chronic degenerative diseases of ageing, is the most prevalent form of arthritis (1) among people aged 40 years or older (2). While the signature pathologic feature of OA is articular cartilage loss, there is growing evidence suggesting that OA is a disease of the whole joint affecting all joint structures, including cartilage, bone, joint capsule, muscles, ligaments and tendons (3-5). Typical presentation of OA includes joint pain and stiffness, leading to a decline in physical functioning. According to the American College of Rheumatology (ACR), OA has been defined as "a heterogeneous group of conditions that lead to joint symptoms and signs, which are associated with defective integrity of articular cartilage in addition to related changes in the underlying bone margins" (6). Although OA is a prevalent condition, the etiology and risk factors have not been fully elucidated. There are few effective medical treatments for OA, with most focusing on the relief of symptoms rather than slowing disease progression (7). Therefore many patients are eventually faced with costly joint replacement as the only option to improve quality of life.

Any joint can be affected by OA. Large weight-bearing joints, the knees and hips, are most commonly affected by OA. Furthermore, OA of knees and hips have the highest clinical impact due to its high prevalence and disabling nature, as they account for more trouble with climbing stairs and walking than any other disease (8). Thus the focus of this thesis will be on the knee and hip OA.

1.2 Burden of osteoarthritis with a focus on knee and hip osteoarthritis

The Global Burden of Disease (GBD) 2010 study reported that musculoskeletal disorders are

the second largest contributor to years lived with disability globally (9). The third most prevalent musculoskeletal disorder in GBD study was OA of the knees and hips (9). In fact, among 291 conditions included in this study, OA of knee and hip was ranked as the 11 highest contributor of global disability (9), resulting in a total of 71.1 million years lived with disability, an increase of 64% from 1990 to 2010 (9) (Figure 1). Knee and hip OA have a profound long-term socioeconomic impact through deteriorating quality of life, especially in terms of pain and functional disability (10). Furthermore, people with OA are twice as likely to have comorbid conditions, such as cardiovascular disease compared to the general population, after adjustment for age, sex and socioeconomic status (11).

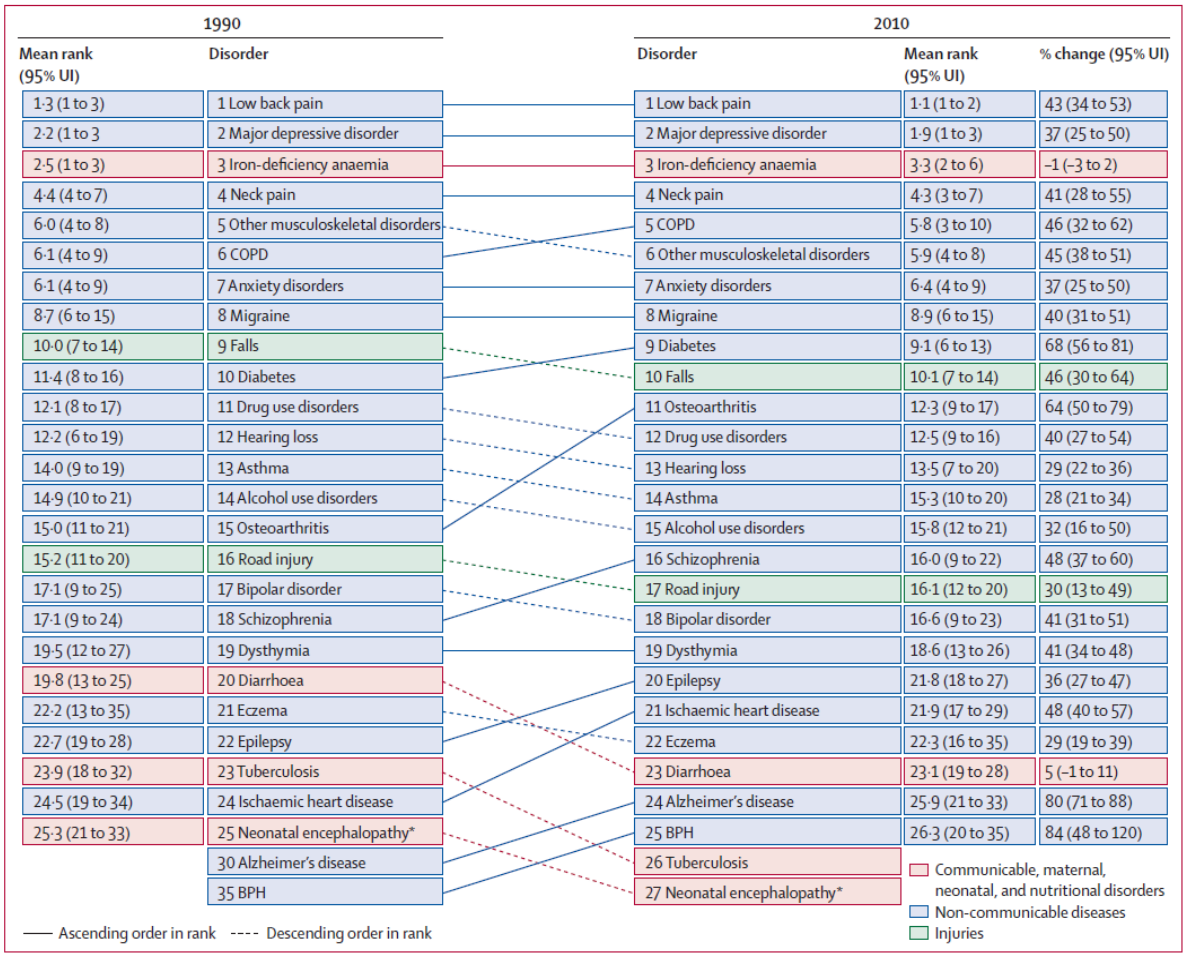

Figure 1: Global years lived with disability (YLDs) ranks with 95% uncertainty intervals (UI) for the 25 most common causes in 1990 and 2010. COPD=chronic obstructive pulmonary disease. BPH=benign prostatic hyperplasia (9).

The expenditure for knee and hip OA is difficult to estimate, as there are direct cost to health services and indirect costs due to loss of productivity and costs of impaired quality of life. The total costs relating to the treatment of OA were estimated at between 1% and 2.5% of the gross domestic product for western countries (3, 12). It was found that, the health care costs for people with knee or hip OA are significantly higher than that for age-matched controls (13, 14). In Australia, OA accounted for $3.28 billion in total health cost in 2012, which was mainly related to joint replacement (15). Since the rate of joint replacement surgery is increasing to improve pain, disability, function, physical activity and quality of life (16) in recent years, it seems reasonable to assume that the total cost associated with knee and hip OA are increasing fast.

1.3 Prevalence of knee and hip osteoarthritis

Depending on the method used to report knee and hip OA, the prevalence varies. Additionally, there is discordance between symptoms and radiographic changes: many people having pathologic and radiographic evidence of knee or hip OA have no symptoms (17). However, the age standardized prevalence is 3.8% for knee OA and 0.85% for hip OA globally with no discernible change from 1990 to 2010 (10). Vulnerability of the knee and hip joints to OA increases with age, and there is clear sex-specific difference. Though before 50 years of age the prevalence of OA is higher in men, after 50 years of age women are more affected by the disease (18, 19). Due to increase in life expectancy and obesity pandemic throughout the world, knee and hip OA will become even more prevalent in future. According to Swedish health register data, currently, one in eight adults aged over 45 years has doctor-diagnosed knee OA, and this had been projected to increase to one in three by the year 2032 (20).

1.4 Clinical manifestations and diagnosis of knee and hip osteoarthritis

Both knee and hip OA are progressive diseases with insidious onset. The major symptom includes localized joint pain (21) generally worsen towards the end of the day, deteriorate with activity and is relieved by rest, while those with severe disease may have pain at rest. Physical findings of knee and hip OA include bony enlargement, crepitus, effusions, joint tenderness, abnormal gait, malalignment and decreased range of motion. The ACR criteria for the diagnosis of both hip and knee OA include a combination of clinical, biochemical and radiographic domains (6, 22).

The ACR clinical and laboratory diagnostic criteria for knee OA include; knee pain plus at least 5 of the following 9 criteria: age >50 years; stiffness <30 minutes; crepitus; bony tenderness; bony enlargement; no palpable warmth; Erythrocyte Sedimentation Rate (ESR) <40 mm/hour; Rheumatoid Factor <1.40; synovial fluid signs of OA (6). Likewise, knee pain plus osteophytes, plus at least 1 of the following 3 criteria: age >50 years; stiffness <30 minutes; crepitus have also been suggested as the clinical and radiographic diagnostic criteria for knee OA (6).

The ACR guidelines for the classification of hip OA comprise pain and either 1) hip internal rotation $\geq 15^0$; pain present on internal rotation of the hip; morning stiffness of the hip for $\leq$60 minutes; age >50 years, or 2) hip internal rotation $<15^0$; ESR $\leq$45 mm/hour. If no ESR was obtained, hip flexion $\leq 115^0$ is substituted (sensitivity 86%, specificity 75%) (22). Patients are also classified to have hip OA if they have pain plus 2 of the following 3 radiographic criteria: osteophytes (femoral or acetabular); joint space narrowing (superior, axial, and/or medial); ESR <20 mm/hour (sensitivity 89%, specificity 91%) (22).

1.5 Treatment of knee and hip osteoarthritis

The aims of both knee and hip OA treatment include alleviation of pain and improvement of function (4), as there is no registered disease modifying drug that can stop the progression of the structural changes within the joint. A combination of non-pharmacological and pharmacological approaches has been suggested as the treatment of these diseases. Non-pharmacologic treatment recommended for the management of knee OA are aerobic, aquatic, and/or resistance exercises, weight loss programs for overweight patients (23).

Pharmacologic treatment for management knee OA included acetaminophen, oral and topical NSAIDs, tramadol, and intra-articular corticosteroid injections; intra-articular hyaluronate injections, duloxetine, and opioids can be conditionally recommended in patients who had inadequate pain relief to initial therapy and has contraindications with joint replacement surgery (23). Though both the non-pharmacologic and pharmacologic treatment recommendations for hip OA were similar to those for the knee OA according to ACR guideline (23), the European League Against Rheumatism (EULAR) guideline suggests that more knowledge on the effectiveness of non-pharmacological treatment is needed in hip OA (24). All these current treatments are symptomatic and limited by side effects or lack of efficacy (5). Regardless of using the available therapies, many people with OA still have significant symptoms and ultimately treated with joint replacement surgery which is thought to be the most effective treatment for knee and hip OA. Better understanding of the disease course and risk factors will offer new approaches of treatment for knee and hip OA, and will help to target the structural change on an individual basis.

1.6 Risk factors for knee and hip osteoarthritis

Recent research in OA has revealed that OA is not a single disease rather a heterogeneous disease and the consequence of different pathological processes resulting in joint failure (25). The common non-modifiable risk factors for both knee and hip OA include age, sex, ethnicity and genetics (3, 26, 27). The common modifiable risk factors include obesity, joint injury, joint alignment, and occupational activity (28, 29). These factors may act by increasing the susceptibility of knee or hip joints to OA, by direct damage to joint tissues or by impairing the process of repair in damaged joint tissue. Additionally, recent epidemiologic studies have shown that knee and hip have different susceptibility to risk factors for OA, suggesting knee and hip OA are different entities and should not be regarded as a unique disease (3).

1.6.1 Osteoarthritis of the knee and hip: associated with different risk factors

As previously mentioned, OA is often the consequence of the interplay between site specific systemic and local factors (30). For example, there are noticeable sex specific differences, with hip OA being more prevalent in men (3, 18) and knee OA more prevalent in women aged over 50 years (18, 19). Knee OA is more related to obesity (31), meta-inflammation produced by adipose cells (32) and metabolic factors (33, 34), while hip OA is mainly linked to bone shape and geometry, such as hip dysplasia (35, 36), abnormal acetabular and femoral shape (35), although there is weak relationship of obesity and hip OA (29). Genetic studies have demonstrated that knee and hip OA have different genetic susceptibility such that a large portion of hip OA and smaller percentage of knee OA are attributable to site specific genes (37). However, to date few studies have examined the risk of knee and hip OA in the same population, which is important to limit the population variation that might confound the results regarding the susceptibility of the joint to different risk factors.

1.6.2 Developments in understanding risk factors for knee and hip osteoarthritis

As there is no registered disease modifying drug to halt the progression of knee and hip OA, treatment of OA to date has relied on the management of symptoms. Over recent years, new approaches and initiatives have been adapted to understanding the disease pathology and mechanisms involved for some of the risk factors.

1.6.2.1 Sex difference in knee and hip osteoarthritis

There are gender differences in the prevalence, incidence and severity of knee and hip OA, especially knee OA affecting more women than men (38). The incidence and severity of OA has been reported to increase particularly after the menopause (38). The reason for this sex difference is poorly understood, but a number of potential mechanisms have been speculated, with most of the studies having focused on sex hormones or alterations in reproductive hormone concentrations that occur with menopause and might contribute to OA pathology. Though several sex hormones, especially the effect of estrogen concentrations have been examined in a number of studies, no clear causal relationship has yet been established (39).

1.6.2.2 Obesity and risk of knee and hip osteoarthritis

Obesity is associated with the risk of knee and hip OA (29, 40, 41). It has been suggested that obesity induced mechanical stresses on cartilage and subchondral bone result in joint damage (33). Obesity induced excessive loading might explain the vulnerability of weight bearing joints such as knee and hip joint to OA, however it is difficult to explain the association between body mass index (BMI) and hand OA (42). Similarly, loss of body fat shows greater symptomatic benefit in knee OA patients than loss of body weight (43). Recent advances in the physiology of adipose tissue add further insights in understanding the relationship between obesity and OA (32). Adipose tissue acts as an endocrine organ by releasing

cytokines, such as interleukins and tumour necrosis factor α (TNF α), as well as adipokines, such as leptin, adiponectin, visfatin and resistin (44). The fact that leptin has a key role in the pathogenesis of OA, started the journey to investigate adipokines as a metabolic link between obesity and OA (33). Although studies indicate that both mechanical and systemic/metabolic factors linked to obesity are the main contributors for OA, the detailed mechanism has not been completely revealed (32).

1.6.2.3 Vitamin D and risk of knee and hip osteoarthritis

Vitamin D is thought to influence the course of OA via its known role in skeletal health specially its effect on bones. Vitamin D plays an essential role in promoting calcium absorption to enable mineralization and promote healthy bones (45). Recent studies has provided the insight that subchondral bone is a target for OA research, as bone marrow lesions (BML) and bone mineral density (BMD) have been associated with both clinical symptoms and structural progression of OA (5, 46). Subtle alterations in joint shape as well as structural and functional alterations in bones may become an important driver in the pathogenesis of OA (47). Recent studies are suggesting that bone is affected at the beginning of the disease and even before cartilage degeneration occurs (35, 48, 49). It has been shown that the change in shape of the bone starts with the change in the microarchitecture of subchondral bone. The microstructure of subchondral bone particularly with respect to the subchondral plate and adjacent trabecular bone differs in osteoarthritic bone compared with non-osteoarthritic bone (50, 51). Though several studies have measured the relationship of serum 25-hydroxy-vitamin D [25(OH)D] and risk of knee OA (52-62), there are not many works that has studied the relationship between the concentration of serum 25(OH)D and risk of hip OA.

1.6.2.4 Birth weight and risk of knee and hip osteoarthritis

Birth weight has been linked to adult health outcome in different populations around the world. For example, low birth weight (LBW) and preterm birth have been associated with hypertension, insulin resistance, cardiovascular disease (63), and more recently reduced bone mass (64). As an underlying mechanism, fetal nutrition in utero leading to reprogramming of the insulin-like growth factor 1 (IGF-1) axis has been proposed (65, 66). IGF-1 stimulates osteoblastic differentiation of mesenchymal stem cells and new bone formation, and thus maintains proper bone microarchitecture and mass (67). Though BMD and bone mineral content has been linked to LBW (68), no previous study has examined the relationship between LBW and OA.

1.7 Themes of the thesis

This thesis will explore four themes: 1) hormonal factors and risk of knee and hip OA, 2) metabolic and vascular factors and risk of knee and hip OA, 3) serum 25(OH)D concentration and risk of hip OA, and 4) birth weight and risk of knee and hip OA.

1.7.1 Sex hormones and risk of knee and hip osteoarthritis

The accelerated vulnerability to OA incidence in postmenopausal women suggests a possible role for sex hormones or the tissue response to sex hormones, particularly oestrogen deficiency, in the systemic predisposition to OA. Majority of the studies to date has examined the association of sex hormones with knee OA (69-75), some with hip OA (76-78) and a couple both knee and hip OA (79, 80). There is no consistent evidence linking estrogen supplementation and risk of OA, as some studies showed beneficial effects of higher level of sex steroids (69, 70, 76, 77) while others have found no association (71, 72, 80), or even an increased risk (78, 79). Some studies examined the relationship between endogenous sex

steroids and the pathogenesis of OA, but the nature of the influence of sex steroids remains uncertain; i.e. endogenous oestradiol (E2) is associated with increased risk of developing radiographic knee OA (73); higher serum concentrations of sex hormone binding globulin (SHBG) associated with increased tibial and patellar cartilage loss (74); no association between cartilage loss and the serum concentrations of dehydroepiandrosterone sulfate (DHEAS), androstenedione (ASD), or testosterone (T) (74); contrasting to the previous two studies a case control study showed middle-aged women with generalized OA had higher circulating concentrations of T and lower circulating concentrations of SHBG (75). A recent systematic review concluded no clear association between female hormones and OA, suggesting that the relationship may be too complex or there are other undetermined aspects that may play a role in the increased incidence of OA in postmenopausal women (81). Circulating sex hormones include DHEAS, ASD, T, E2 and oestrone sulphate. SHBG is a transport protein for androgens and oestrogens in the circulation as a result availability of concentration of sex hormones depends on the concentration of SHBG. However, none of these aforementioned studies have examined all the sex hormones and SHBG in the same population.

The ratio of the length of the index (2D) and ring (4D) fingers (expressed as 2D:4D) reflects the effects of prenatal sex steroids on 19 skeletogenic genes (82) and skeletogenic SMOC1 gene (83), suggesting a possible underlying genetic determinant of 2D:4D. Exposure to *in utero* higher parental testosterone and lower estrogen concentrations results in low 2D:4D (84-87) and there are always consistent finding that men have a lower average 2D:4D than women (84, 85). A number of phenomena for example sexual ability, facial shape, physical and athletic ability, performance in examinations, myocardial infarction have been linked to the reduction of 2D:4D (88). OA can be related to 2D:4D through physical activity or through

hormonal factors. Previous studies of 2D:4D and the risk of knee OA have yielded inconclusive results; with two case-control studies showing a linear relationship between lower 2D:4D and increased risk of knee OA (88, 89) and one cross-sectional study found no association between 2D:4D with risk of knee OA (90). The only case control study examining the relationship between 2D:4D with risk of hip OA found inconsistent association (88). There is no longitudinal study that showed the relationship between 2D:4D and risk of knee or hip OA.

1.7.2 Metabolic and vascular factors and risk of knee and hip osteoarthritis

The metabolic syndrome (MetS) is defined as the clustering of abdominal obesity, hypertension, dyslipidaemia and hyperglycaemia (91). Obesity is the central component of MetS. Though biomechanical loading exerted by obesity is one of the important aspect in the OA pathogenesis, recent advances in the physiology of adipose tissue add further insights in understanding the relationship between inflammatory and metabolic aspect of obesity and OA (32).

Another MetS component hypertension, is more prevalent among the individuals with knee OA (31, 34, 92, 93), and hip OA (31) compared to non-OA individuals in observational studies. The common association between hypertension and knee and hip OA might be linked to obesity as previous studies have proven that obesity is a strong determinant of hypertension (94, 95). However, most of the studies that showed a positive association between hypertension and OA have not adjusted for obesity (34, 92, 93). In other studies, where obesity has been adjusted, the association between hypertension and knee and hip OA disappeared (31).

Histological changes due to lipid deposition in chondrocytes at the early stage of OA initiates the hypothesis that lipid deposition in the joint might trigger the pathophysiological process of OA development (96). Although the exact details of the mechanisms remain obscure, it seems likely that altered lipid metabolism plays a role in the development of OA evidenced from epidemiologic studies that have indicated association between elevated serum cholesterol and knee OA (97-99). Additionally from the finding of the Chingford study the association of increased serum cholesterol with risk of knee OA was independent of obesity (98).

The result regarding hyperglycaemia and OA remained inconclusive, as five small scale studies (100-104) concluded a positive association between increased blood glucose levels and incidence of OA, but the two large studies (40, 105) reported no association or merely an insignificant association between OA and hyperglycaemia. The definitions of OA in these studies were not homogeneous, some has defined OA, OA in any site (40, 100, 101, 103, 105), knee and hip OA(102, 104). Another point that should be taken into account is that the aforementioned studies have used different cut-off points for defining hyperglycaemia, for example diabetes instead of hyperglycaemia (92, 104, 106), measured HbA1c (34, 107), others depended on self-reported diabetes (40).

MetS and OA shares some common risk factors i.e. age and obesity. A large body of evidence suggests that various interrelated metabolic factors contribute to OA (33, 108). Among the participants of NHANES III survey, not only the individual components of the MetS were more prevalent in people with knee OA but also MetS was found to be 5.26 times more common (92). Similarly, the 3 year follow up of the Research on Osteoarthritis / Osteoporosis Against Disability (ROAD) study showed an increased association between

components of MetS with the incidence and progression of radiological knee OA in a Japanese population (93). However, both of these studies are confounded by obesity which is the major driver of knee OA. The Malmö Diet and Cancer study reported only central obesity to be associated with increased risk of knee OA independent of BMI, and neither MetS nor components of MetS to be associated with hip OA (31). Taken together, a positive signal for an independent correlation between knee OA and MetS independent of obesity potentially exists.

The components of MetS are prevalent among the patients of vascular disease (109) and perhaps, therefore, vascular changes may influence the pathology of OA. Cardiovascular risk factors (110), and vascular comorbidities (111, 112) are highly prevalent among the patients of OA compared with people without OA. The cardiovascular risk factors increase the production proinflammatory cytokines and adipokines (33) which are the main characteristics of systemic inflammation (113). Chronic systemic inflammation via the production of interleukin-6 and TNF α might also initiate or stimulate the OA process (114). People with cardiovascular disorders always have generalized changes in vascular architecture (115). Besides, multiple small bone infarcts are common in advanced OA (116).

Increased intima media thickness of the carotid artery, a subclinical marker of large vessel atherosclerosis, has been shown to be associated with increased prevalence of knee OA in women (106), and increased popliteal artery wall thickness, has been shown to be associated with generalized OA defined as OA at multiple sites, including knee or hip (117). These findings suggest that established macrovascular disease is involved in the pathogenesis of knee OA. However, little work has examined the role of microcirculation in the pathogenesis of OA.

1.7.3 Serum concentrations of 25(OH)D and risk of hip osteoarthritis

OA is a complex disorder involving multiple tissue and cell types. Despite known risk factors such as age, gender, obesity growing evidence suggests a role of the bone microenvironment, including osteoblast differentiation, the process of mineralization, and bone remodeling leading to change in the shape of the bone (118). For example, in a case control study it was found that a significant change occurred in shape of the proximal femur among hip OA participants from baseline to follow-up compared to non-hip OA controls (119). The form of deformities that have been linked to hip OA are pistol grip deformity and an abnormally low neck shaft angle (120), femoro-acetabular impingement, cam type deformity and pincer deformity (35), a deep acetabular socket (121).

Serum 25(OH)D has been linked to changing the bone microstructure; bone mass and/or BMD (122), which can change the shape and geometry of the bone (46). Owing to the role of vitamin D in bone and cartilage turnover and the importance of systematic and local bone changes in OA (123) it has been hypothesized that serum 25(OH)D might be associated with OA. Provided that hip is a bony joint, bony deformity related to change in bone shape will be more pronounced to hip OA. There are few studies examining the association between serum 25(OH)D and hip OA. However, these studies yielded inconclusive results as some studies have reported that low serum 25(OH)D concentrations are associated with an increased prevalence and incidence of hip OA (54, 124), others have shown no association (57, 58). The studies on serum 25(OH)D and hip OA had a number of limitations which make interpretation of the findings difficult, including small sample size (54, 58, 124), cross-sectional study design (124), and failure to adjust for all of the confounders (54, 124). As a result the role of serum 25(OH)D concentrations in the pathogenesis of OA remained unanswered.

1.7.4 Birth weight and risk of knee and hip osteoarthritis

Several adulthood diseases have their deep rooted link to the in utero programming. For example hypertension, insulin resistance, cardiovascular disease (63), and more recently reduced bone mass (64) have been linked to LBW and preterm birth. As an underlying mechanism reprogramming of the insulin-like growth factor 1 (IGF-1) axis has been proposed (66, 125). Bone mass and especially bone mineral contents, which can change the bone shape are highly associated with birth weight independent of maternal factors such as gestational age, maternal smoking and nutrition, and are largely mediated by skeletal size and particularly adult height (68). Preterm babies are found to develop a postural deformation of the legs with wide hip abduction and external rotation giving a 'frog leg' posture that persists till the age of 3-4.5 years (126). They also have high frequency of unstable hip type IIc (risky) and IId (decentralized) (127). Developmental abnormalities during infancy and childhood leave the joint with unusual shape, especially in the hip. However, clinical screening of developmental dysplasia of the hip (DDH) is insufficient for early diagnosis and decision about the treatment of premature babies owing to the unstable hip (127).

1.8 Knowledge gap

OA affects more women than men after the age of 50 years suggests that this may be due to deficiency in available circulating sex hormone. However, several studies has examined some of the circulating sex hormones and SHBG or estrogen supplements with the relationship of knee and hip OA and had inconclusive results. All the sex hormones originate from DHEA and convert to ASD then from ASD to T, oestrone sulphate or E2 and might have either direct effect or might act after conversion. As a result it is important to examine all the sex hormones and SHBG all together. However, all the previous studies failed to achieve this. Likewise, the few studies investigating the relationship between 2D:4D with risk of knee and

hip OA resulted in inconclusive findings and to date no longitudinal study has been carried out to confirm the relationship.

While age, gender, obesity, injury, ethnicity, occupational activity, and genetics are the established risk factors for both knee and hip OA, it is increasingly recognized that other systemic risk factors are responsible for knee or hip OA susceptibility. Recent epidemiologic studies are suggesting a major driver of the development and progression of OA is related to systemic inflammation present in MetS. Although there is emerging evidence suggesting a role of vascular pathology in the pathogenesis of OA, little work has examined the role of microcirculation, such as the retinal vascular caliber.

Owing to the role of vitamin D in bone health and the importance of bone changes in OA it is possible that serum 25(OH)D might be associated with the risk of OA. However evidence from epidemiological studies with regard to the association between serum 25(OH)D concentrations and the risk of hip OA is conflicting.

Evidence is also emerging that OA of knee and hip may be susceptible to LBW and preterm birth. Though bone mass and bone mineral contents has been highly associated with low birth weight and preterm birth has been linked to postural deformity and unstable hip, no previous work has been done to examine the role of birth weight and preterm birth and the risk of knee and hip OA.

Knee and hip OA are susceptible to different risk factors. However, the evidence for heterogeneity in susceptibility of these two conditions to different risk factors came from studies that have examined knee OA and hip OA in different population. It is important to

examine both knee and hip OA in the same population to avoid the population variation that might confound the results.

1.9 Joint replacement for osteoarthritis as a surrogate marker of severe knee and hip osteoarthritis

OA is a continuum form normal through to clinical OA through to the end stage joint disease i.e. joint replacement or arthroplasty. In OA research many different definitions of disease have been used. These include self-reported OA, symptomatic OA using questionnaire without a confirmed diagnosis, through to studies examining imaging modalities such as X-ray or more recently MRI. Each of these has potential advantages and disadvantages.

Joint replacement or arthroplasty for OA has been used as a surrogate marker to define OA in many studies (27-29, 31, 128). Although definition of joint replacement or arthroplasty due to OA only identifies the tip of the iceberg of the true problem, it is a valid mechanism of defining severe symptomatic OA. This measure has the advantage of connecting the problem with the disease burden and economic burden of OA. Using joint replacement for OA as a surrogate for severe OA, several risk factors for OA have been identified, such as ethnicity (27), obesity (29, 128), occupational activity (28, 129). In Australia there is universal health cover as a result joint replacement is accessible to the whole population. In this context in a previous study, it has been showed that independent of confounders, knee structural change was a risk factor for knee replacement (130)

A potential strength of using joint replacement to address the questions listed above is that there are a number of major cohort studies with prospectively collected data as well as the Australian Orthopaedic Association National Joint Replacement Registry (AOANJRR) where

there is comprehensive collection of knee and hip replacements. Thus this combination of resources provides a unique opportunity to address some of the above questions.

1.10 Aims of the thesis

This thesis aims to identify novel and systemic risk factors that are associated with the incidence of knee and hip replacement for OA in large linkage cohort studies, in an attempt to identify specific factors which are associated with symptomatic end-stage knee and hip OA that required joint replacement surgery.

This thesis had 4 major themes. In particular, this thesis aimed to examine:

1. The association between hormonal factors, including circulating sex hormones and 2D:4D ratio (an indicator of *in utero* exposure to sex hormones), and risk of knee and hip replacement for OA;
2. The association between metabolic and vascular factors and risk of knee and hip replacement for OA;
3. The association between serum 25(OH)D concentration and the risk of hip replacement for OA;
4. The association between LBW and preterm birth and risk of knee and hip replacement for OA.

Chapter 2: Methods and study populations

2.1 Study design: linkage cohort study

Obtaining health outcomes through data linkage between existing cohort studies and health services or disease registries is a valid, cost-effective method and has been used in several large scale epidemiological studies (128, 131-133). In the process of data linkage, common identifiers are used to link the cohort data with health services or disease register, for example to identify whether study participants have been admitted to hospital, or had any procedure (133, 134). Record linkage acts as a passive follow-up, so even if participants have been lost to follow-up from the study, the participants' specific health outcomes can still be obtained (131, 134). Linkage of cohort data with health service datasets has been routine in several countries such as Sweden (128, 135), the United Kingdom (133, 136), and Canada (137) for many years.

Deterministic and probabilistic methods are the two main methods of data linkage. Deterministic linkage links two records based on complete agreement between the common identifiers (134). Mechanism involved in the probabilistic record linkage involves linking records based on the statistical probability that common identifiers belong to the same person (131). It is ideal to link data with domestic linkage where accuracy of linking is an important issue and where data quality within the various datasets is high (134). In this thesis data linkage was done for two large cohort studies, the Melbourne Collaborative Cohort Study (MCCS), and the Australian Diabetes, Obesity and Lifestyle (AusDiab) Study, linked to the AOA NJRR.

For linking the MCCS with AOA NJRR direct linkage method was used by identifying exact matches and then probabilistic matches were reviewed. The matching was performed on these data using U.S. Bureau of the Census Record Linkage software. Identifying information for MCCS participants, including first name, last name, date of birth and gender, was provided to the AOA NJRR in order to identify those participants who had had a primary or revision joint replacement between 1 January 2001 (commencement of Victorian data collection) and 31 December 2011. The AOA NJRR forwarded this information to the MCCS, which was added to the MCCS database. The data linkage was approved by the Cancer Council Victoria Human Research Ethics Committee and Monash University Human Research Ethics Committee.

The same procedure was done to link the AusDaib study with the AOA NJRR. Matching of AusDiab participants was done using first name, surname, date of birth, and gender, to the AOA NJRR between 1 January 2002 (commencement of national data collection) and 31 December 2011. The AOA NJRR forwarded this information to the AusDiab, which was added to the AusDiab database. The data linkage study was approved by the Alfred Hospital Ethics Committee, the University of Adelaide and Monash University Human Research Ethics Committees.

2.2 The Melbourne Collaborative Cohort study (MCCS)

MCCS is a prospective cohort study of 41,514 people (17,045 men, 24,469 women) aged between 40 and 69 years at recruitment. The participants for the MCCS study were recruited via the Electoral Rolls, advertisements, and community announcements in local media from the metropolitan area of Melbourne, Victoria, Australia, between 1990 and 1994. To increase the variability in lifestyle and genetic factors, the study recruited a quarter of the participants

who were migrants to Australia from Italy and Greece. The main focus of the study was to identifying risk factors for cancer, type 2 diabetes, cardiovascular disease, eye disease and arthritis (138). The information regarding demographic and lifestyle factors, including date of birth, country of birth, smoking, highest level of education, and physical activity were collected using questionnaires. Height, weight, waist circumference, blood pressure, and 2D:4D were measured using standard protocols. Serum biomarkers, including lipid, glucose and hormones, were measured from stored blood samples. This study protocol was approved by the Cancer Council Victoria Human Research Ethics Committee. Participants gave written consent to participate and for the investigators to obtain access to their medical records.

2.3 The Australian Diabetes, Obesity and Lifestyle study (AusDiab)

AusDaib study is a prospective cohort study of 11,247 people (44.9% were male) aged over 25 years. Participants for AusDiab study were recruited by a stratified cluster sampling method, involving seven strata (six states and the Northern Territory) and clusters based on census collector districts, during 1999-2000 (139). For the AusDiab study, follow-up data was collected between 2004 and 2005. AusDiab study has all the information regarding general demographics - age, sex, ethnicity; life-style related factors - general health and well-being, alcohol/tobacco use, physical activity; physical measurements – height, weight, body fat, blood pressure, blood measurements, retinal vascular measures and serum 25(OH)D. During the follow-up between 2004 and 2005, AusDiab study also collected data on birth weight and preterm birth. The study was approved by the International Diabetes Institute Ethics Committee and the Monash University Human Research Ethics Committee.

2.4 Australian Orthopaedic Association National Joint Replacement Registry (AOA NJRR)

The Commonwealth Department of Health and Ageing funded Australian Orthopedic Association in 1998 to establish the National Joint Replacement Registry (140). The AOA NJRR inaugurated on 1 September 1999 and began data collection in South Australia followed by a staged implementation in each of the Australian states and territories (140). AOA NJRR started collecting data fully nationally during 2002 (140). The purpose of the registry is to define, improve and maintain the quality of care of individuals receiving joint replacement surgery. It achieves this by collecting a defined minimum data set that enables outcomes to be determined on the basis of patient characteristics, prosthesis type and features, method of prosthesis fixation and surgical technique used (140). In Australia the performance and outcome of both hip and knee replacement surgery is monitored by the registry. AOA NJRR is able to determine the success or complications by using detailed matching technology. The registry receives cooperation for data collection from all hospitals both public and private undertaking joint replacement surgery. The registry validates its data by using both internal systems and external data sources which includes state and territory health department data. The validation process is done using a sequential multilevel matching process. The validation process resulted in over 93% of Registry records verified against health department data (140). AOA NJRR has detailed information on prostheses, patient demographics, type of joint replacement (i.e. whether a first joint replacement or a revision) and reason for joint replacement (i.e. for OA or other reason) (140). The Registry is able to obtain an almost complete dataset relating to hip and knee replacement, following the retrieval of unreported records and checking of unmatched data.

Chapter 3: Hormonal factors and risk of knee and hip osteoarthritis

Given the increased susceptibility to OA incidence in postmenopausal women, a possible role for sex hormones has been suggested as a risk factor of OA (3, 73). However, a recent systematic review concluded that there is no clear association between sex hormones and OA, or the relationship of sex hormones are too complex (81). The studies that examined circulating sex hormones and SHBG with the relationship of knee and hip OA have not examined all the sex hormones and SHBG all together in the same population. The few studies investigating the relationship between 2D:4D, a marker of *in utero* sex hormone exposure, and risk of knee and hip OA resulted in inconclusive findings, with two case-control studies showing a linear relationship between lower 2D:4D and increased risk of knee OA (88, 89) and one cross-sectional study found no association between 2D:4D and risk of knee OA (90).

This chapter addresses questions related to the relationship between circulating sex hormones and SHBG, and 2D:4D with risk of severe knee and hip OA. This chapter incorporates two publications; one examining circulating sex hormones and SHBG and joint replacement for severe knee and hip OA, and the other examining 2D:4D and joint replacement for severe knee and hip OA.

Declaration for Thesis Chapter 3

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

Manuscript: Incidence of total knee and hip replacement due to osteoarthritis in relation to circulating sex steroid hormone concentrations in women. Arthritis & rheumatology 2014; 66(8):2144-51.

Nature of contribution	**Extent of contribution (%)**
Data analysis and interpretation, manuscript development and preparation	70%

The following co-authors contributed to the work. Where co-authors are students at Monash University, the extent of their contribution in percentage terms is stated:

Name	**Nature of contribution**	**Extent of contribution (%) for student co-authors only**
Cicuttini	Study design, data analysis and interpretation, and manuscript editing	
Bell	Data analysis and interpretation, and manuscript editing	
Robinson	Data analysis and interpretation, and manuscript editing	
Davis	Data analysis and interpretation, and manuscript editing	
Giles	Study design, data collection and manuscript editing	
Graves	Data acquisition and manuscript editing	
Milne	Data analysis and interpretation and manuscript editing	
Wang	Study design, data analysis and interpretation, and manuscript editing	

The undersigned hereby certify that the above declaration correctly reflects the nature and extend o the candidate's and co-author' contributions to this work

Candidate's Signature		**Date**
Main Supervisor's Signature		**Date**

Declaration for Thesis Chapter 3

Declaration by candidate

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

Manuscript: Association between index-to-ring finger length ratio and risk of severe knee and hip osteoarthritis requiring total joint replacement. Rheumatology (Oxford) 2014; 53(7):1200-7.

Nature of contribution	Extent of contribution (%)
Data analysis and interpretation, manuscript development and preparation	70%

The following co-authors contributed to the work. Where co-authors are students at Monash University, the extent of their contribution in percentage terms is stated:

Name	Nature of contribution	Extent of contribution (%) for student co-authors only
Wang	Study design, data analysis and interpretation, manuscript development and preparation	
Muller	Data collection and manuscript editing	
Wluka	Data interpretation and manuscript editing	
Giles	Study design, data collection, and manuscript editing	
Manning	Data interpretation and manuscript editing	
Graves	Data acquisition and manuscript editing	
Cicuttini	Study design, data analysis and interpretation, and manuscript editing	

The undersigned hereby certify that the above declaration correctly reflects the nature and extend of the candidate's and co-author' contributions to this work

Candidate's Signature		**Date**
Main Supervisor's Signature		**Date**

3.1 Circulating sex steroid hormone and risk of knee and hip osteoarthritis

There is no consistent evidence linking estrogen supplementation and risk of OA, as some studies showed beneficial effects (69, 70, 76, 77, 79) while others found no association (71, 72, 80), or even an increased risk (78). The studies examining the relationship between endogenous sex steroids and the pathogenesis of OA, also reported inconclusive results. For example, lower E2 (73) and higher SHBG (74) were associated with increased risk of developing knee OA; there was no association between knee cartilage loss and serum concentrations of DHEAS, ASD, or T (74); middle-aged women with generalized OA had higher concentrations of T and lower concentrations of SHBG (75). Only two studies examined the relationship between knee and hip OA and hormone replacement therapy, with one showing no beneficial effect (80) and the other one showing detrimental effect (79). However, one of these studies have combined knee and hip OA together (80). As previously mentioned, none of these studies have examined all the sex hormones in the same population.

Given the inconsistency in the previous results and paucity of research examining all the circulating sex hormones, this longitudinal study explored the relationship between circulating concentrations of sex steroids (DHEAS, Ad, T, E2, oestrone sulphate) and SHBG, and the incidence of total knee and hip replacement for OA in a large prospective cohort study.

Hussain SM, Cicuttini FM, Bell RJ, Robinson PJ, Davis SR, Giles GG, Graves S, Milne RL, Wang Y. Incidence of total knee and hip replacement due to osteoarthritis in relation to circulating sex steroid hormone concentrations in women. Arthritis & rheumatology; 2014; 66(8):2144-51.

In this study it was found, lower E2 concentration is a risk factor for knee OA; lower ASD concentration and higher SHBG concentration are risk factors for hip OA in women. The findings suggest a role of circulating sex steroids in the pathogenesis of OA and that knee and hip OA are susceptible to different hormones. Modifying these steroid concentrations may provide potential strategies for the prevention and treatment of knee and hip OA.

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Incidence of Total Knee and Hip Replacement for Osteoarthritis in Relation to Circulating Sex Steroid Hormone Concentrations in Women

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Objective. **The increased prevalence of osteoarthritis (OA) in postmenopausal women suggests that changes in either circulating sex steroid concentrations or the tissue response to sex steroids may have a role in the pathogenesis of OA. The aim of this study was to examine whether circulating sex steroid concentrations are associated with the incidence of total knee and total hip replacement for OA.**

Methods. **Study subjects (n = 2,621; all women) were recruited in 1990–1994 from the Melbourne Collaborative Cohort Study (MCCS). Circulating sex steroid concentrations were measured in blood samples obtained from the women at the time of recruitment. The incidence of total knee and total hip replacement for OA during 2001–2011 was determined by linking the MCCS records to the Australian Orthopaedic Association National Joint Replacement Registry.**

Results. **During the followup period, 115 women had undergone total knee replacement and 99 had undergone total hip replacement for OA. Greater log-transformed concentrations of estradiol were associated with a lower incidence of knee replacement (hazard ratio [HR] 0.70, 95% confidence interval [95% CI] 0.50–0.96), and greater log-transformed concentrations of androstenedione were associated with a lower incidence of hip replacement (HR 0.70, 95% CI 0.52–0.93). In contrast, greater log-transformed concentrations of sex hormone binding globulin (SHBG) were associated with a higher incidence of hip replacement (HR 1.70, 95% CI 1.05–2.77).**

Conclusion. **A lower estradiol concentration is a risk factor for knee OA, while a lower androstenedione concentration and higher SHBG concentration are risk factors for hip OA in women. These findings suggest that circulating sex steroids have a role in the pathogenesis of OA, and that modifying these steroid concentrations may provide a potential strategy for the prevention and treatment of knee and hip OA.**

Osteoarthritis (OA) is a major health problem associated with significant morbidity and disability, particularly in patients with OA of the knees and hips. In 2010, a total prevalence of 71.1 million years lived with disability was attributed to OA as the cause, an increase of 64% since 1990 (1). Currently, there are no officially

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approved disease-modifying drugs that slow the progression of OA. Understanding the role of modifiable factors in the pathogenesis of OA is important for the prevention and treatment of the disease. In individuals older than age 50 years, OA is more common among women than among men (2). The increased prevalence of OA in postmenopausal women suggests that changes in either circulating sex steroid concentrations or the tissue response to sex steroids may modulate the disease process and play a role in the development of OA.

The findings from epidemiologic studies with regard to the risk of OA in relation to reproductive history and use of oral contraceptives and postmenopausal exogenous hormones are conflicting. Some studies have shown beneficial effects of exogenous steroids (3–7), while others have shown no effect (8–10) or even detrimental effects (11). Some studies examined the relationship between endogenous sex steroids and the pathogenesis of OA (12–14), but the nature of the influence of sex steroids remains uncertain. In the Southeast Michigan Arthritis Cohort, women having lower baseline concentrations of endogenous estradiol (E_2) had an increased risk of developing radiographic knee OA (12). A cross-sectional study of asymptomatic women found an association between higher serum concentrations of sex hormone binding globulin (SHBG) and increased tibial and patellar cartilage loss, whereas no association was observed between cartilage loss and the serum concentrations of dehydroepiandrosterone sulfate (DHEAS), androstenedione (ASD), or testosterone (13). In contrast, a case–control study showed that middle-aged women with generalized OA had higher circulating concentrations of testosterone and lower circulating concentrations of SHBG when compared with controls (14). In a recent systematic review, no clear association between female hormones and OA was observed, suggesting that the relationship may be too complex or that other undetermined aspects may play a role in the increased incidence of OA in postmenopausal women (15).

Studies exploring the association between sex steroids and knee or hip OA have generally defined OA on the basis of findings from various imaging techniques (12,13). Another method for defining OA is based on the need for joint replacement (4,16). This latter definition signifies the presence of severe knee and hip OA, which is relevant to the symptomatic disease burden and to health economics. There is increasing evidence suggesting that hip OA and knee OA are susceptible to different risk factors (17,18). For example, bone shape and geometry are important in the etiology of hip OA (19). These factors, although also important at the knee, have a lesser role, when compared with that of soft tissue integrity, in the pathogenesis of knee OA (17). We therefore examined the relationship between circulating concentrations of sex steroids (DHEAS, ASD, testosterone, E_2, and estrone sulfate) and SHBG and the incidence of total knee and total hip replacement for OA in a large prospective cohort study.

PATIENTS AND METHODS

Cohort. The Melbourne Collaborative Cohort Study (MCCS) is a prospective cohort study of 41,514 participants (including 24,469 women) ages 27–75 years (99.3% being ages 40–69 years) at baseline (20). Participants were recruited between 1990 and 1994 via the electoral roll, advertisements, and community announcements in local media. Southern European migrants to Australia were deliberately oversampled to extend the range of lifestyle exposures and to increase genetic variation. The study protocol was approved by the Human Research Ethics Committee of Cancer Council Victoria. Subjects gave written consent to participate and to allow investigators to obtain access to their medical records. Followup was conducted by record linkage to the electoral roll, electronic telephone books, and the Victorian Cancer Registry and death records. From 2003 onward, 28,046 study participants (68% of the original MCCS participants) took part in the second round of followup.

Study participants. Of the recruited participants, 2,584 (6.2%) were excluded for one of the following reasons: died or left Australia prior to January 1, 2001; in the second round of followup, reported having undergone any joint replacement prior to January 1, 2001; left Australia before the recorded date of the joint replacement; or had revision joint replacement as the first recorded procedure within the study period (21). Of the remaining 38,930 participants, 23,275 were women. The current study was restricted to the 2,668 women whose blood samples were assessed for sex steroid concentrations; the samples were obtained at the time of recruitment (1990–1994) and were stored in liquid nitrogen until used. These women, as compared with women whose blood had not been assessed for sex steroid concentrations, were older (mean ± SD 55.3 ± 8.9 years versus 54.7 ± 8.5 years; $P < 0.001$), were less likely to be born in Australia or the United Kingdom (75.7% versus 77.7%; $P = 0.02$), and had a higher body mass index (BMI) (mean ± SD 27.2 ± 5.2 kg/m^2 versus 26.6 ± 4.8 kg/m^2; $P < 0.001$).

Demographic factors and anthropometric measures. At baseline, demographic factors, including date of birth and country of birth (Australia, United Kingdom, Italy, or Greece), were collected from face-to-face interviews. Data on use of menopausal hormone replacement therapy and oral contraceptives were collected using a questionnaire. Height and weight were measured according to written protocols based on standard procedures. Weight was measured to the nearest

0.1 kg, using a digital electronic scale; height was measured to the nearest 1 mm, using a stadiometer. BMI was calculated as weight (in kg) divided by height (in m^2).

Serum analysis. Serum concentrations of E_2, estrone sulfate, testosterone, DHEAS, ASD, and SHBG were measured by the same person. All analytes were measured between 6 years and 13 years after the collection of blood samples (median 9 years) (22).

To avoid the potential for differential measurement error due to batch-to-batch variability in hormone measurements, samples were randomly assigned to batches. In each batch, 10% of the samples were aliquots from pooled plasma that had been stored with the samples from participants. Testosterone and E_2 concentrations were measured by electrochemiluminescence immunoassay (Elecsys 2010 analyzer; Roche Diagnostics). The lower limits of detection (LLDs) were 18 pmoles/liter for E_2 and 0.1 nmoles/liter for testosterone. The coefficients of variation (CVs) were 10% (8% within batches and 6% between batches) for E_2 at a concentration of 157 pmoles/liter and 7% (4% within batches and 5% between batches) for testosterone at a concentration of 4.3 nmoles/liter. Estrone sulfate was measured by radioimmunoassay (RIA) (DSL-5400; Beckman Coulter), with an LLD of 0.03 nmoles/liter and CV of 15% (13% within batches and 8% between batches) at a concentration of 5.7 nmoles/liter. DHEAS was measured by competitive immunoassay (Immulite Analyzer; DPC), with an LLD of 0.2 μmoles/liter and CV of 10% (9% within batches and 6% between batches) at a concentration of 4.0 μmoles/liter. ASD was analyzed by RIA (DSL-4200; Beckman Coulter), with an LLD of 0.02 nmoles/liter and CV of 15% (11% within batches and 9% between batches) at a concentration of 2.6 nmoles/liter. SHBG was measured by immunometric assay (Immulite Analyzer; DPC), with an LLD of 2 nmoles/liter and CV of 7% (6% within batches and 4% between batches) at a concentration of 45.0 nmoles/liter (22).

A reliability study was performed before commencement of the study to determine the reliability of the estrone sulfate, testosterone, DHEAS, ASD, and SHBG measurements. Plasma samples from 45 women who had provided blood at baseline and again ~1 year later were divided into 2 aliquots and measured in separate batches one week apart. The intraclass correlation coefficient (ICC) was calculated as the proportion of the total variance due to variation between persons, in which the total variance included components due to between-persons, between-sampling occasions, and residual variance. The ICCs were 0.85 (95% confidence interval [95% CI] 0.78–0.92) for estrone sulfate, 0.65 (95% CI 0.52–0.77) for testosterone, 0.87 (95% CI 0.81–0.93) for DHEAS, 0.61 (95% CI 0.44–0.78) for ASD, and 0.90 (95% CI 0.85–0.95) for SHBG. There were insufficient samples to perform an equivalent reliability study for E_2 (22).

Identification of total knee and total hip joint replacements. Cases comprised women who underwent either a total hip replacement or a total knee joint replacement, each of which was identified from the Australian Orthopaedic Association (AOA) National Joint Replacement Registry (NJRR). The Registry began data collection in September 1999 and implementation was introduced in a staged manner in each of the Australian States and Territories. The Registry commenced data collection in Victoria in January 2001, and the AOA NJRR has collected national data on joint replacement procedures performed in Australia since 2002 (23). The Registry monitors hip and knee joint replacements. It has detailed information on the joint replacement prostheses, patients' demographics, the reason for joint replacement, whether the procedure is a primary joint replacement or a revision, and the type of revision. Data are collected from both public and private hospitals, and these data are validated using a sequential multilevel matching process against State and Territory Health Department unit record data (24). Following the validation process and retrieval of unreported records, the Registry provides an almost complete set of data with regard to hip and knee replacements in Australia (24).

Identifying information on each MCCS participant, including first name, last name, date of birth, and sex, was provided to the staff at the AOA NJRR in order to identify those MCCS participants who had undergone a joint replacement between January 1, 2001 and December 31, 2011. Data matching was performed using US Bureau of the Census record linkage software. Exact matches were identified, and probabilistic matches were reviewed. The staff from the AOA NJRR forwarded this information to the MCCS and it was then added to the MCCS database. The data linkage of the AOA NJRR with the MCCS was approved by the Human Research Ethics Committees of Cancer Council Victoria and Monash University.

Definition of knee and hip OA. Knee OA and hip OA were defined as the first total knee replacement or total hip replacement attributed to a contemporaneous diagnosis of OA, as recorded in the AOA NJRR (21). If a person had undergone multiple joint replacements (bilateral knee replacement, bilateral hip replacement, or both knee and hip replacement), only the first procedure recorded in the AOA NJRR was considered.

Statistical analysis. Analysis of variance was used to compare mean values, the chi-square test was used to compare proportions, and the Kruskal-Wallis test was used to compare concentrations of steroids and SHBG among groups. The values for all sex steroid concentrations and the SHBG concentration were natural log transformed, because none of these data appeared to be normally distributed.

Cox proportional hazards regression models were used to estimate the hazard ratio (HR) for the likelihood of an association between the first recorded total knee replacement or total hip replacement attributable to OA and the concentration of each sex steroid and SHBG. The followup period for total joint replacement (i.e., calculation of person-time) began on January 1, 2001 and ended on the date of first total joint replacement for OA or date of censoring. Participants were censored on the date of first total joint replacement performed for indications other than OA, the date of death, the date the participant left Australia, or the end of followup (i.e., December 31, 2011, the date that ascertainment of joint replacement by the NJRR was complete), whichever came first. Each analysis was adjusted for age, country of birth, and BMI, because these are the risk factors for total joint replacement in this population (21,25). To test whether any of the associations

of steroid concentrations or SHBG concentrations with joint replacement risk were modified by obesity (defined as a BMI $\geq$30 kg/m^2), interactions were fitted and tested using the likelihood ratio test.

Tests based on Cox regression methods showed no evidence that proportional hazards assumptions were violated for any analysis. All statistical analyses were performed using Stata software version 12.0 (StataCorp).

RESULTS

Among the participants at baseline, 47 women reported having received exogenous sex steroid therapy. These subjects were excluded, thus leaving 2,621 women in the final analysis (Figure 1). A total of 214 total joint replacements (115 knee replacements and 99 hip replacements) performed for OA were identified from January 1, 2001 to December 31, 2011. The mean ± SD followup time was 9.7 ± 2.9 years.

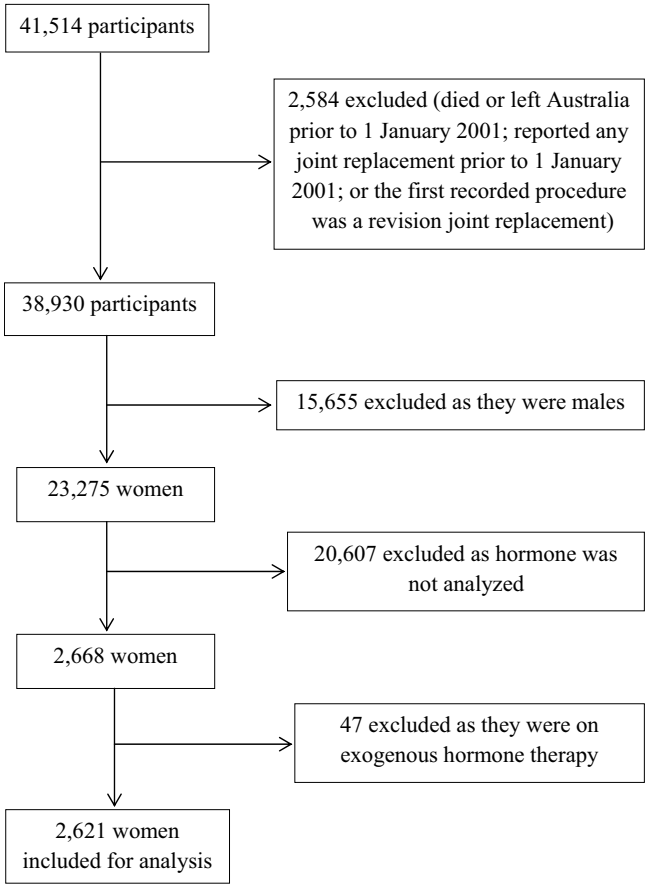

Figure 1. Flow chart depicting the distribution of recruited study participants and reasons for exclusion from the study.

Characteristics of the study participants are presented in Table 1. Women who had received a total joint replacement, compared with those who had not undergone a total joint replacement, were older and more likely to be born in Australia or the United Kingdom. In addition, women who underwent a total knee replacement had greater BMI than those with no history of joint replacement. Those who had undergone a total joint replacement had a lower median serum concentration of ASD, estrone sulfate, and E_2 compared with those who had not undergone a joint replacement. Finally, women who had undergone a total hip replacement had a higher median concentration of SHBG compared with the other groups.

Univariate analyses revealed that the log-transformed concentrations of ASD, E_2, estrone sulfate, and SHBG were inversely associated with the incidence of total knee replacement (Table 2). When the analysis was adjusted for age, country of birth, and BMI, only the log-transformed concentration of E_2 remained associated with a lower incidence of total knee replacement (HR 0.70, 95% CI 0.50–0.96).

In univariate analyses assessing the risk of total hip replacement, the log-transformed concentrations of DHEAS, ASD, E_2, and estrone sulfate were inversely associated with the incidence of total hip replacement (Table 3). When the analysis was adjusted for age, country of birth, and BMI, the log-transformed concentration of ASD was still associated with a lower incidence of total hip replacement (HR 0.70, 95% CI 0.52–0.93). In contrast, in adjusted analyses, a greater log-transformed concentration of SHBG was positively associated with a higher incidence of total hip replacement (HR 1.70, 95% CI 1.05–2.77).

In all analyses, there was no evidence that obesity modified the associations between the sex steroid concentrations or SHBG concentration and the risk of total knee or total hip joint replacement (each $P > 0.10$).

DISCUSSION

To our knowledge, this prospective cohort study is the first to explore the possibility of a relationship between circulating concentrations of endogenous sex steroids or SHBG and the likelihood of total knee or total hip joint replacement for OA in women. The log-transformed concentration of E_2 was inversely associated with the incidence of total knee replacement, and the log-transformed concentration of ASD was inversely associated with the incidence of total hip replacement.

Table 1. Characteristics of the study population*

	Total knee replacement (n = 115)	Total hip replacement (n = 99)	No joint replacement (n = 2,407)	P†
Age at baseline, mean ± SD years	58.9 ± 6.5	59.1 ± 8.0	55.0 ± 8.9	<0.001
Age at TJR, mean ± SD years	72.3 ± 6.1	72.5 ± 8.5	–	
BMI, mean ± SD kg/m^2	31.3 ± 6.0	27.5 ± 5.2	27.5 ± 5.2	<0.001
Born in Australia or United Kingdom, no. (%)	85 (73.9)	75 (75.8)	1,647 (68.4)	0.001
Oral contraceptive use, no. (%)				0.62
Never	56 (48.7)	46 (46.9)	1,071 (44.5)	
Former	59 (51.3)	52 (53.1)	1,333 (55.4)	
HRT use, no. (%)				0.99
Never	103 (89.6)	88 (89.8)	2,124 (89.4)	
Former	12 (10.4)	10 (10.2)	253 (10.6)	
Steroid or SHBG concentration, median (IQR)				
DHEAS, μmoles/liter	1.7 (1.2–2.3)	1.6 (0.9–2.6)	1.9 (1.1–3.0)	0.01
ASD, nmoles/liter	2.5 (1.7–3.6)	2.4 (1.5–3.2)	3.0 (2.0–4.2)	<0.001
Testosterone, nmoles/liter	0.8 (0.5–1.1)	0.8 (0.5–1.1)	0.8 (0.5–1.2)	0.81
E_2, pmoles/liter	67.5 (49.5–98.0)	67.5 (50.0–100.0)	74.0 (53.0–234.0)	<0.001
Estrone sulfate, nmoles/liter	4.4 (3.6–6.5)	4.1 (3.1–6.6)	5.3 (3.4–9.1)	<0.001
SHBG, nmoles/liter	42.7 (29.8–58.2)	55.7 (41.7–70.6)	51.7 (37.8–69.1)	<0.001

* TJR = total joint replacement; BMI = body mass index; HRT = hormone replacement therapy; SHBG = sex hormone binding globulin; IQR = interquartile range; DHEAS = dehydroepiandrosterone sulfate; ASD = androstenedione; E_2 = estradiol.
† For difference among the 3 groups.

In contrast, the log-transformed concentration of SHBG was positively associated with the incidence of total hip replacement.

The observed association between incident total knee replacement for OA and a lower E_2 concentration was independent of age, country of birth, and BMI, each of which is an established risk factor for knee OA (21,25). Consistent with our findings, middle-aged women in the Southeast Michigan Arthritis Cohort who developed radiographically defined knee OA had a significantly lower baseline serum concentration of E_2 (12). In a cross-sectional study of postmenopausal women, those receiving long-term estrogen therapy had greater knee cartilage volume compared with those not receiving such therapy (3). In mice, E_2 was found to preserve the articular cartilage and subchondral bone (26). Taken together, these findings suggest that E_2 has a role in protecting against the development of knee OA.

The results of the present study did not reveal a relationship between the endogenous E_2 concentration and the incidence of total hip replacement. No previous study has examined this, but in a cross-sectional analysis

Table 2. Relationship between sex steroid and SHBG concentrations and the incidence of total knee replacement for osteoarthritis*

	Univariate analysis		Multivariate analysis†	
	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
DHEAS	0.82 (0.65–1.04)	0.10	1.15 (0.87–1.51)	0.33
ASD	0.71 (0.55–0.90)	0.005	0.80 (0.59–1.08)	0.15
Testosterone	0.90 (0.69–1.16)	0.42	0.95 (0.73–1.25)	0.74
E_2	0.63 (0.50–0.79)	<0.001	0.70 (0.50–0.96)	0.03
Estrone sulfate	0.73 (0.56–0.93)	0.01	0.91 (0.66–1.25)	0.56
SHBG	0.46 (0.32–0.67)	<0.001	0.98 (0.64–1.51)	0.93

* All hormone concentrations are natural log transformed. SHBG = sex hormone binding globulin; 95% CI = 95% confidence interval; DHEAS = dehydroepiandrosterone sulfate; ASD = androstenedione; E_2 = estradiol.
† Adjusted for age, body mass index, and country of birth.

Table 3. Relationship between sex steroid and SHBG concentrations and the incidence of total hip replacement for osteoarthritis*

	Univariate analysis		Multivariate analysis†	
	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
DHEAS	0.70 (0.54–0.89)	0.004	0.92 (0.69–1.22)	0.58
ASD	0.62 (0.49–0.77)	<0.001	0.70 (0.52–0.93)	0.01
Testosterone	0.88 (0.66–1.16)	0.37	1.00 (0.75–1.34)	0.99
E_2	0.65 (0.52–0.83)	<0.001	0.79 (0.58–1.08)	0.14
Estrone sulfate	0.59 (0.44–0.78)	<0.001	0.75 (0.53–1.06)	0.10
SHBG	1.31 (0.85–2.03)	0.22	1.70 (1.05–2.77)	0.03

* All hormone concentrations are natural log transformed. SHBG = sex hormone binding globulin; 95% CI = 95% confidence interval; DHEAS = dehydroepiandrosterone sulfate; ASD = androstenedione; E_2 = estradiol.
† Adjusted for age, body mass index, and country of birth.

of 4,366 women ages ≥65 years who were recruited for the Study of Osteoporotic Fractures, women who were receiving E_2 hormone replacement therapy had a reduced risk of hip OA (odds ratio 0.62, 95% CI 0.49–0.86) (6). Further longitudinal work will be needed to confirm whether exogenous E_2 has a beneficial effect on the hip joints or whether a bias toward healthy women may have influenced these findings.

We found that a higher serum concentration of ASD was associated with a reduced incidence of total hip replacement, but not total knee replacement. ASD is an important precursor for estrone (E_1), E_2, and testosterone production in nongonadal tissues (27). ASD increases bone mineral density at both the metaphyseal and diaphyseal regions of the femur in rats (28,29). ASD increases the quality and quantity of bone mineral in both cancellous and cortical bone in rats and monkeys (30). Whether these effects are a direct action of ASD or whether they may be attributed to conversion of ASD to testosterone, E_1, or E_2 within the bone has not yet been elucidated.

A higher concentration of SHBG, which is a transport protein for androgens and estrogens in the circulation, was associated with an increased risk of total hip replacement. SHBG binds E_2 and ASD with high affinity (31). Therefore, lower SHBG concentrations will result in higher fractions of free sex steroids, whereas higher concentrations of SHBG will result in lower concentrations of unbound sex steroids (31). Thus, the relationship between a high SHBG concentration and the risk of joint replacement may reflect lower concentrations of unbound sex steroids in the circulation, particularly ASD and E_2. In a cohort of women ages 33–77 years, the SHBG concentration was inversely associated with bone mineral density (32). It may be that a higher concentration of ASD and lower concentration of SHBG will reduce the risk of hip OA and subsequent need for hip joint replacement, through both anabolic and antireabsorptive actions.

The results of the present study also demonstrated a decreased level of E_2 as a risk factor for knee OA, and both a decreased level of ASD and increased level of SHBG as risk factors for hip OA, suggesting that the pathogenesis of knee OA and hip OA may differ. Factors affecting soft tissue integrity and neuromuscular control of the knee for its stability play an important role in the pathogenesis of knee OA, whereas E_2 has greater influences (3,33–35). It has been shown that E_2 exerts either direct or indirect effects on the maintenance and well-being of skeletal muscle and cartilage through estrogen receptor α (ERα) and ERβ, via several pathways such as transforming growth factor β and the insulin-like growth factor 1 (IGF-1) and IGF-2 pathways (3,33,34). It has also been found that E_2 plays an important role in fibroblast metabolism (35). In contrast, bony shape and joint congruence appear to have a greater role in the development of hip OA (19). Bony shape and joint congruence depend on osteoclast formation and bone resorption, which are more likely to be affected by the direct and indirect inhibitory effects of testosterone (36). The fact that a lower ASD concentration and higher SHBG concentration are risk factors for hip OA is consistent with this androgen drive. In cultured osteoblast cells, ASD converts into testosterone and dihydrotestosterone through the 17β-hydroxysteroid dehydrogenase pathway (37), which affects bone maturation and maintains the homeostasis of mature bone (37,38).

We did not observe an association between the incidence of total joint replacement for OA and the concentrations of testosterone, DHEAS, or estrone sulfate. With regard to testosterone, this result might be attributable to the imprecision of the assay used, since the concentrations of this sex steroid are low in women (39). Our findings are consistent with the results of a cross-sectional study of 176 women ages 40–67 years, in which there was no relationship observed between the endogenous testosterone concentration and knee structure (13). In contrast, a case–control study of 77 women ages 41–52 years showed that a higher serum testosterone concentration was associated with increased risk of generalized OA (14). This was probably because the design of that study differed, and because the study was underpowered and did not adjust for confounders. Estrone sulfate is a reservoir for E_1 and E_2 in the circulation, and similarly, DHEAS is a reservoir for circulating DHEA, which is a precursor for the production of ASD, E_1, and E_2. The lack of an association between the estrone sulfate or DHEAS concentrations and outcomes in the joints in relation to OA does not detract from the potential clinical significance of our other findings.

The major strengths of our study include its large sample size, prospective design, and inclusion of a large number of participants from different ethnic backgrounds. Moreover, the AOA NJRR data are validated and nearly complete regarding joint replacements in Australia (24). We examined the association between circulating sex steroid concentrations and OA of the knee and hip in the same population. We carefully excluded from our study those women who were receiv-

ing any systemic exogenous steroid therapy at baseline. During the first round of followup in 1995–1998 and second round of followup in 2003–2007, the participants were asked about any history of exogenous steroid therapy; the number of women who reported having received exogenous steroid therapy was negligible (31 of 2,366 in the first round and 4 of 1,860 in the second round). The results did not vary substantially after excluding these individuals from the analysis (results not shown).

The findings of the present study need to be considered within the context of its limitations. The blood samples were collected at various times throughout the day, and therefore the circadian rhythms in hormone secretion may have been a source of variance (40). We did not examine free E_2 and free testosterone. Although the free levels of these hormones can be calculated (41), we did not believe that an analysis of the free hormone levels would have added value to the current analysis, since the assays used for the measurement of total E_2 and total testosterone were not highly precise. The blood samples were collected at the time of recruitment into the MCCS (1990–1994), whereas the NJRR-linked joint replacement data were available from January 1, 2001 onward. We did not have complete and reliable joint replacement data for the study participants prior to 2001. However, this is likely to have resulted in nondifferential misclassification of joint replacement status in relation to the steroid and SHBG measurements, and thus may have attenuated any of the observed associations.

Furthermore, using total joint replacement to define OA provides only partial evidence of the true problem, because the use of total joint replacement as the treatment for OA could be influenced by a number of factors, such as access to health care, physician bias, and patient-level factors, in addition to disease severity (42). The associations persisted after adjusting for age, country of birth, and BMI, and we restricted the study to women only. Although it is possible that residual confounding persists, it is unlikely that socioeconomic differences alone would explain these findings.

Our findings indicating that a lower E_2 concentration is a risk factor for knee OA and that a lower ASD concentration and greater SHBG concentration are risk factors for hip OA in women suggest that the role of these sex steroids in the pathogenesis of knee and hip OA needs further exploration. Moreover, an approach aimed at modifying the serum concentrations of these sex steroids and SHBG may provide a potential strategy for the prevention and treatment of large-joint OA.

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AUTHOR CONTRIBUTIONS

All authors were involved in drafting the article or revising it critically for important intellectual content, and all authors approved the final version to be published. Dr. Wang 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.

Study conception and design. Cicuttini, Giles, Wang.

Acquisition of data. Giles, Graves.

Analysis and interpretation of data. Hussain, Cicuttini, Bell, Robinson, Davis, Giles, Milne, Wang.

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3.2 Index-to-ring finger length ratio (a marker of sex hormones in-utero) and risk of knee and hip osteoarthritis

2D:4D has been linked to *in utero* hormone exposure such that, higher parental testosterone and lower estrogen concentrations results in a lower 2D:4D (84-87). As 2D:4D is related to physical and athletic ability (88), it may contribute to knee and/or hip OA through physical activity or hormonal factors. Previous studies yielded inconclusive results; showing a linear relationship between lower 2D:4D and increased risk of knee OA (88, 89), or no association between 2D:4D and risk of knee OA (90). Only one study examined the relation of 2D:4D with risk of hip OA and reported an inconsistent association (88). Till to date no longitudinal study has examined the relationship between 2D:4D with risk of knee and hip OA.

This longitudinal study was aimed to determine whether 2D:4D was associated with the risk of severe knee and hip OA requiring knee or hip joint replacement.

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This study demonstrated that, lower 2D:4D is associated with an increased risk of severe knee OA requiring knee joint replacement. This is not observed for severe hip OA. These results may be explained in part by joint injuries associated with high-level physical activity in those with lower 2D:4D and the greater susceptibility of knee OA in response to injury than hip OA; they may also reflect hormonal influences on the growth of bone, cartilage and soft tissue.

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Original article

Association between index-to-ring finger length ratio and risk of severe knee and hip osteoarthritis requiring total joint replacement

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Abstract

Objective. The data are conflicting for the association between the index-to-ring finger length ratio (2D:4D) and the risk of OA. The aim of this cohort study was to examine the relationship between 2D:4D and the risk of severe knee and hip OA requiring total joint replacement.

Methods. A total of 14 511 participants in the Melbourne Collaborative Cohort Study had 2D:4D assessed from hand photocopies. The incidence of total knee replacement and total hip replacement between 2001 and 2011 was determined by linking the cohort records to the Australian Orthopaedic Association National Joint Replacement Registry.

Results. Over an average 10.5 years of follow-up, 580 participants had total knee replacement and 499 had total hip replacement. Greater right 2D:4D [hazard ratio (HR) 0.91 for a S.D. increase in 2D:4D, 95% CI 0.84, 0.99, $P = 0.03$] and average right and left 2D:4D (HR 0.91 for a S.D. increase in 2D:4D, 95% CI 0.84, 0.99, $P = 0.02$) were associated with a reduced incidence of total knee replacement. These associations persisted when participants whose fingers had any features that might have affected the validity of 2D:4D measurements were excluded. No significant associations were observed between 2D:4D and the incidence of total hip replacement.

Conclusion. A lower 2D:4D is associated with an increased risk of severe knee OA requiring total knee replacement, but not the risk of severe hip OA. The underlying mechanisms for the association warrant further investigation.

Key words: index-to-ring finger length ratio, osteoarthritis, total knee replacement, total hip replacement.

CLINICAL SCIENCE

Introduction

OA is a major public health problem with significant morbidity and disability associated with OA of the knees and hips [1]. Hormonal factors are thought to play a role in the pathogenesis of OA, and possibly account for some of the well-described sex differences in the prevalence of OA [2, 3].

Anthropological studies suggest that there are consistent sex differences in the ratio of the lengths of the index (2D) and ring (4D) fingers (expressed as 2D:4D) such that men have a lower average 2D:4D than women [4, 5]. 2D:4D has been suggested as a proxy indicator of prenatal testosterone levels, with low 2D:4D reflecting higher *in utero* testosterone exposure [4–7]. There is evidence that 2D:4D reflects the effects of prenatal sex steroids

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on 19 skeletogenic genes [8] and 2D:4D is strongly associated with the skeletogenic *SMOC1* gene [9], suggesting a possible underlying genetic determinant of 2D:4D.

Previous studies of 2D:4D and the risk of knee OA have yielded inconsistent results. These are summarized in Table 1. While two case–control studies showed a linear relationship between lower 2D:4D and increased risk of radiographic knee OA [10, 11] and one cross-sectional study found type 3 finger length pattern (longer ring finger than index finger) to be associated with increased risk of total knee replacement (TKR) for OA [12], the Framingham study found no association between 2D:4D and the risk of radiographic knee OA [13]. There are more consistent data for the association between 2D:4D and the risk of hip OA. One case–control study found no significant association between 2D:4D and the risk of radiographic hip OA [10], and a recent cross-sectional study showed no association between finger length patterns and the risk of total hip replacement (THR) for OA [12]. Due to the nature of these studies, being case–control or cross-sectional, temporal relationships cannot be examined. Identification of the association between 2D:4D and OA risk from large cohort studies would provide stronger evidence and thus increase understanding of disease pathogenesis.

One method for defining OA is to use total joint replacement, which signifies severe clinical knee and hip OA relevant to the symptomatic disease burden and economic impact of OA [14, 15]. Thus the aim of this study was to determine whether 2D:4D was associated with the risk of severe knee or hip OA requiring TKR or THR in a large cohort study.

Patients and methods

Study participants

The Melbourne Collaborative Cohort Study (MCCS) is a prospective cohort study of 41 514 people (24 469 women) between 27 and 75 years of age (99.3% age 40–69 years) at baseline [16]. Participants were recruited via the electoral rolls (enrolment to vote is compulsory for Australian adults), advertisements and community announcements in local media between 1990 and 1994 in the Melbourne metropolitan area. All participants provided written informed consent and the study protocol was approved by the Cancer Council Victoria Human Research Ethics Committee.

Participants who attended face-to-face follow-up between 2003 and 2009 and had their hands photocopied were eligible for inclusion in this study ($n = 14\,917$); 406 (3%) were excluded from the current analysis because they died or left Australia before 1 January 2001, reported a joint replacement performed before 1 January 2001 at the MCCS follow-up, left Australia before the date of a primary joint replacement or had a revision surgery as the first procedure recorded in the Australian Orthopaedic Association National Joint Replacement Registry (AOA NJRR). These exclusions left 14 511 participants available for analysis.

Demographic, lifestyle factors and physical measurements

At baseline, information was obtained on demographic and lifestyle factors, including date of birth, country of birth (Australia, United Kingdom, Italy, Greece), smoking status, alcohol consumption, physical activity during leisure time and highest level of education. Height and weight were measured according to written protocols using standard procedures [17]. Weight was measured to the nearest 0.1 kg using digital electronic scales and height was measured to the nearest 1 mm using a stadiometer. BMI was calculated as weight in kilograms divided by the square of height in metres.

Measurement of 2D:4D

During the face-to-face follow-up conducted during 2003–9, participants had their hands photocopied for the purpose of measuring 2D:4D. The length of the index and ring fingers were measured from photocopies of the surface of the hand using vernier callipers with a resolution of 0.01 mm [18]. Measurements were taken from the tip of the finger to the basal crease. Where two creases were visible at the base of the digit, the crease proximal to the palm was chosen. The length of the index finger was divided by the length of the ring finger to obtain the 2D:4D. Any features that made measurement difficult or might have affected the validity of the measurements, such as finger deformities potentially due to hand arthritis or injuries, were also recorded. The measurement was undertaken by a team of trained research assistants at Cancer Council Victoria. One hundred photocopies were measured by each research assistant twice to assess the inter- and intraobserver reliability of the digit measurements. Inter- and intraobserver reliability was high for raw digit measurements, with intraclass correlation coefficients (ICCs) for left and right index and ring fingers all being >0.95. The ICCs for 2D:4D were slightly lower than those for raw digit measurements (0.80 for right and 0.73 for left 2D:4D), but still suggest that the observed variability in digit ratio is largely due to between-individual differences rather than measurement error [18].

Identification of incident primary knee and hip joint replacement

Cases were identified from the AOA NJRR. The registry began data collection in September 1999, with staged implementation across the Australian states and territories. Victoria commenced data collection in 2001, and national data on arthroplasty procedures in Australia is available from 2002 [19]. Hip and knee joint replacements are monitored with detailed information available on prostheses, demographics, reasons for revisions and types of revision. Data are collected from both public and private hospitals and validated using a sequential multilevel matching process against State and Territory Health Department unit record data. Following the validation process and retrieval of unreported records, the registry collects an almost complete set of data relating to hip and knee replacement in Australia [20].

Table 1 Association between 2D:4D and OA, data from three published studies

Author and year	Study design	Participants	Measurement of exposure	Measurement of outcome	Main results
Zhang *et al.* 2008 [10]	Case–control study	2049 cases with symptomatic knee or hip OA seen by an orthopaedic surgeon or rheumatologist. 1123 controls undergoing i.v. urography	Visual classification of finger patterns. 2D:4D ratio measured from radiographs of both hands by taking the mean of the ratio of the right and left hand	Radiography of the knee before knee joint replacement due to OA. Radiography of the pelvis for hip OA	The type 3 finger pattern (longer ring finger than index finger) was associated with knee OA, and the risk was greater in women. There was a linear relationship between both the 2D:4D ratio and the risk of knee OA. The risk of hip OA was inconsistent
Ferraro *et al.* 2009 [11]	Nested case–control study from the Clearwater OA Study	236 cases with knee OA. 236 controls randomly selected	Visual classification of finger patterns. 2D:4D ratio measured from digitized films of hand radiographs	Radiographic knee OA	The type 3 finger pattern was associated with knee OA. Women demonstrated a stronger association of visual type 3 finger pattern and knee OA
Haugen *et al.* 2011 [13]	Cross-sectional study	1039 participants from the Framingham community cohort	Right 2D:4D phalangeal and metacarpal bones measured from hand radiographs. Left hand was measured if measurement of the right hand was not possible.	Radiographic knee OA. Self-reported knee injury. Meniscal lesions from MRI	There were no significant associations between 2D:4D and radiographic knee OA, severe symptomatic knee OA or meniscal lesions

TKR: total knee replacement; 2D:4D: index-to-ring finger length ratio.

Identifying information for MCCS participants, including first name, last name, date of birth and gender, was provided to the AOA NJRR in order to identify those participants who had had a primary or revision joint replacement between 1 January 2001 (commencement of Victorian data collection) and 31 December 2011. The matching was performed on these data using U.S. Bureau of the Census Record Linkage software. Exact matches were identified and probabilistic matches were reviewed. The AOA NJRR forwarded this information to the MCCS, which was added to the MCCS database. The data linkage was approved by the Cancer Council Victoria Human Research Ethics Committee and Monash University Human Research Ethics Committee.

Statistical analysis

Cox proportional hazards regression models were used to estimate the hazard ratio (HR) and 95% CI for the first recorded TKR and THR associated with each 2D:4D measurement, with age as the time scale. Follow-up for TKR and THR (i.e. calculation of person-time) began at 1 January 2001 and ended at the date of the first TKR or THR for OA or the date of censoring. Participants were censored at either the date of the first TKR or THR for indications other than OA, the date of death, the date they left Australia or the end of follow-up (i.e. 31 December 2011, when ascertainment of joint replacement by AOA NJRR was complete), whichever came first.

The right and left 2D:4D were examined separately. Due to the strong correlation between the right and left 2D:4D ($r = 0.58$ in this sample), the average of the right and left 2D:4D was also examined. 2D:4D was examined as a standardized continuous variable (measured 2D:4D divided by the sex-specific S.D. of the ratio). 2D:4D was also categorized into approximate sex-specific tertiles based on the analysis sample. The highest tertile was used as the referent category. Linear associations between the 2D:4D and the risk of TKR and THR were investigated by comparing regression models with the 2D:4D as a categorical variable and a pseudo-continuous variable (using the median value in each 2D:4D category) using the likelihood ratio test. Tests for trends across categories of the 2D:4D (calculated using the 2D:4D as a pseudo-continuous variable) were presented only when there was no statistical evidence of a departure from a linear association with the risk of joint replacement. To assess whether associations between 2D:4D and the risk of total joint replacement were modified by sex or country of birth, interaction terms between sex or country of birth and 2D:4D were fitted and models with and without these interaction terms were compared using the likelihood ratio test. Sensitivity analyses were also conducted excluding any participants whose fingers had features that might have affected the validity of the measurements. All analyses were adjusted for BMI and country of birth and stratified by sex.

Tests based on Schoenfeld residuals and graphical methods using Kaplan–Meier curves showed no evidence that proportional hazard assumptions were violated. All statistical analyses were performed using Stata/IC 12.1 (StataCorp, College Station, TX, USA).

Results

Over the 10.5 years (S.D. 1.7) of follow-up, 580 incident TKRs and 499 incident THRs were identified. The characteristics of the study participants are shown in Table 2. When the 2D:4D ratios were examined as continuous variables, greater right and average 2D:4D was associated with a reduced incidence of TKR (HR 0.91, 95% CI 0.84, 0.99 for both) with no significant association observed for left 2D:4D (Table 3). When the 2D:4D ratios were examined as categorical variables, there was a linear trend for an association between left and average 2D:4D and the incidence of TKR, with a lower ratio being associated with an increased risk of TKR. For left 2D:4D, the HR was 1.15 (95% CI 0.93, 1.42) for middle tertile and 1.28 (1.04, 1.57) for the lowest tertile. For average 2D:4D, the HR was 1.01 (95% CI 0.82, 1.26) for middle tertile and 1.29 (1.05, 1.59) for the lowest tertile. No significant associations were observed between 2D:4D and the incidence of THR. There was no evidence that sex or country of birth

Table 2 Characteristics of study participants

	TKR ($n = 580$)	THR ($n = 499$)	Without knee or hip replacement ($n = 13\,432$)
Age at study entry (1990–4), years	57.7 (7.1)	57.5 (7.5)	53.8 (8.3)
Female, *n* (%)	356 (61.4)	314 (62.9)	7928 (59.0)
BMI at study entry, kg/m^2	29.5 (4.8)	27.3 (4.6)	26.7 (4.2)
Country of birth, *n* (%)			
Australia/UK	470 (81.0)	426 (85.4)	9603 (71.5)
Italy/Greece	110 (19.0)	73 (14.6)	3829 (28.5)
Right 2D:4D	0.950 (0.042)	0.952 (0.038)	0.956 (0.037)
Left 2D:4D	0.957 (0.040)	0.960 (0.037)	0.961 (0.037)
Average 2D:4D	0.954 (0.035)	0.956 (0.033)	0.958 (0.033)

Data presented as mean (S.D.) unless otherwise indicated. 2D:4D: index-to-ring finger length ratio; TKR: total knee replacement; THR: total hip replacement.

modified the association between 2D:4D and the incidence of TKR or THR.

There were 830 participants whose fingers had features that might have affected the validity of the measurements. Sensitivity analyses were performed by excluding these participants (Table 4). Among the remaining 13 681 participants, there were 524 TKRs and 454 THRs. The estimates were similar to those obtained from the complete sample. Greater right (HR 0.89, 95% CI 0.81, 0.97, $P = 0.01$) and average (HR 0.90, 95% CI 0.83, 0.99, $P = 0.03$) 2D:4D were associated with a reduced incidence of TKR, with consistently significant associations when the ratios were examined in tertiles. For right 2D:4D, the HR was 0.97 (95% CI 0.77, 1.21) for the middle tertile and 1.29 (1.05, 1.60) for the lowest tertile. For average 2D:4D, the HR was 0.98 (95% CI 0.78, 1.23) for the middle tertile and 1.30 (1.05, 1.61) for the lowest tertile. No significant association was seen for left 2D:4D and TKR. There were no significant associations between 2D:4D and the incidence of THR.

There was no association between 2D:4D and participation in vigorous physical activity at recruitment. Including physical activity in the regression models did not alter the results (data not shown).

Table 3 Association between 2D:4D and risk of total knee or hip replacement for OA ($n = 14\,511$)

	TKR		THR	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Right 2D:4D	0.91 (0.84, 0.99)	0.03	0.95 (0.86, 1.03)	0.21
Tertile 3	1		1	
Tertile 2	0.94 (0.76, 1.16)		1.15 (0.91, 1.44)	
Tertile 1	1.20 (0.98, 1.46)	0.06*	1.25 (1.00, 1.56)	0.05*
Left 2D:4D	0.93 (0.86, 1.01)	0.09	0.99 (0.91, 1.08)	0.84
Tertile 3	1		1	
Tertile 2	1.15 (0.93, 1.42)		0.92 (0.74, 1.14)	
Tertile 1	1.28 (1.04, 1.57)	0.02*	0.95 (0.77, 1.18)	0.65*
Average 2D:4D	0.91 (0.84, 0.99)	0.02	0.97 (0.89, 1.06)	0.49
Tertile 3	1		1	
Tertile 2	1.01 (0.82, 1.26)		0.98 (0.78, 1.22)	
Tertile 1	1.29 (1.05, 1.59)	0.01*	1.12 (0.90, 1.39)	0.30*

2D:4D: index-to-ring finger length ratio; TKR: total knee replacement; THR: total hip replacement; HR: hazard ratio. All models adjusted for BMI and country of birth and stratified by sex. **P* for trend.

Table 4 Association between 2D:4D and risk of total knee or hip replacement for OA in sensitivity analyses ($n = 13\,681$)

	TKR		THR	
	HR (95% CI)	*P*-value	HR (95% CI)	*P*-value
Right 2D:4D	0.89 (0.81, 0.97)	0.01	0.94 (0.86, 1.04)	0.22
Tertile 3	1		1	
Tertile 2	0.97 (0.77, 1.21)		1.11 (0.88, 1.41)	
Tertile 1	1.29 (1.05, 1.60)	0.01*	1.23 (0.98, 1.55)	0.08*
Left 2D:4D	0.94 (0.87, 1.03)	0.20	0.98 (0.89, 1.07)	0.65
Tertile 3	1		1	
Tertile 2	1.10 (0.88, 1.37)		0.93 (0.74, 1.17)	
Tertile 1	1.21 (0.98, 1.51)	0.08*	0.97 (0.77, 1.21)	0.77*
Average 2D:4D	0.90 (0.83, 0.99)	0.03	0.96 (0.87, 1.06)	0.40
Tertile 3	1		1	
Tertile 2	0.98 (0.78, 1.23)		0.93 (0.74, 1.18)	
Tertile 1	1.30 (1.05, 1.61)	0.01*	1.11 (0.88, 1.40)	0.34*

2D:4D: index-to-ring finger length ratio; TKR: total knee replacement; THR: total hip replacement; HR: hazard ratio. All models adjusted for BMI and country of birth and stratified by sex. **P* for trend.

Discussion

To our knowledge, this is the first cohort study examining the relationship between 2D:4D and the incidence of severe OA requiring TKR or THR. We found that lower 2D:4D was associated with an increased incidence of TKR but not THR.

We showed that lower 2D:4D was associated with an increased incidence of TKR when the ratio was examined on either the right or left hand or the average. The findings were consistent with those from two previous case–control studies that showed an association between lower 2D:4D and an increased risk of radiographic knee OA [10, 11] when 2D:4D was measured from hand radiographs using the average ratio of both hands [10] or the ratio of the right hand [11]. Although there is no cohort study examining the temporal relationship between 2D:4D phalangeal ratios and hand OA, the findings from the Framingham OA study suggest that hand OA may contribute to a lower 2D:4D phalangeal ratio, as the index finger is more frequently affected by OA than the ring finger, due to joint space narrowing, bone attrition, malalignment or deformity [21]. Neither of the two case–control studies took into account whether the participants had hand OA, which might be an important confounder [10, 11], whereas in our study the associations for right and average 2D:4D persisted when participants with finger deformities potentially due to arthritis or injury were excluded from analysis. We observed a stronger association of right 2D:4D than left 2D:4D with TKR risk. This is supportive of this being a real effect, as this pattern is common in 2D:4D work and there is evidence that right 2D:4D is inversely associated with prenatal exposure and sensitivity to testosterone, with weak evidence for any association with left 2D:4D [4, 22]. Our findings were also supported by a recently published cross-sectional study showing that type 3 finger length pattern was associated with an increased risk of TKR for OA, with the results persistent in sensitivity analyses of participants with no evidence of hand OA on photographs [12]. However, in this study finger length ratios were assessed visually on photographs of both hands with no actual measurement of finger length, which is prone to misclassifications of finger length pattern [12]. In contrast, the Framingham study, which assessed 2D:4D on right hand radiographs, did not find an association between 2D:4D and a risk of knee OA [13]. A significant difference with our study was that we examined severe knee OA as defined by needing a TKR, whereas the Framingham study used radiographic knee OA as the outcome measure, which may have reduced the sensitivity for detecting this relationship. In support of this, the Framingham study found a non-significant trend for lower 2D:4D to be associated with an increased risk of knee OA in sensitivity analyses of men without evidence of hand OA [adjusted odds ratio (OR) 1.76, 95% CI 0.87, 3.57, P for trend = 0.11] [13]. The Framingham study was cross-sectional and had a modest sample size, which may have reduced its power to show an effect.

While the Framingham study found an association between lower 2D:4D and an increased risk of knee injury [13], we did not find an association between 2D:4D and participation in vigorous activity, or evidence to suggest that the association between 2D:4D and the risk of knee OA can be explained by physical activity. This finding is consistent with the findings of the Genetics of Osteoarthritis and Lifestyle Study [10]. Although there is some evidence that sporting ability and achievement in sports and athletics are negatively related to 2D:4D [23], this might not reflect levels of regular physical activity in the general population. In our study, the measure of physical activity did not directly assess sporting ability, nor did the measure report past physical activity, which may also be important in this regard.

We found an increased incidence of severe knee OA but no association for severe hip OA in relation to lower 2D:4D. This is consistent with the findings from the Genetics of Osteoarthritis and Lifestyle Study [10] and the Age, Gene/Environment Susceptibility (AGES) Reykjavik study [12], which found that measured 2D:4D or visually assessed finger length pattern was related to knee OA but not hip OA. The site difference in OA risk in relation to 2D:4D may be explained, at least in part, by the different susceptibility of knee and hip OA in response to injury. The Johns Hopkins Precursors Study, a prospective study conducted in medical students, revealed that early knee injuries were associated with a 2.95 (95% CI 1.35, 6.45) times relative risk of symptomatic knee OA, with no association between hip injuries and later development of hip OA [24]. Moreover, this study reported higher prevalence and incidence of knee injury than hip injury [24], indicating that the knee is more prone to injury than the hip. There is evidence for effects of hormones on the growth of bone, cartilage and soft tissue [25, 26]. It may be that the differences seen in the relationship between 2D:4D and the risk of knee and hip OA reflect the increasingly described differences in the mechanisms of disease in the two joints [27, 28].

Our study has several strengths and limitations. The large size of the population-based cohort with complete follow-up in terms of total joint replacement since 2001 and inclusion of participants from different ethnic backgrounds are the major strengths. Digit measurements were made with a high degree of reliability by trained research assistants. Additionally we examined the relationship between 2D:4D and the risk of knee and hip OA in the same population, for right and left 2D:4D separately, and demonstrated that the mechanism of disease differs in these joints. One of the limitations is that we used photocopies of the hand, which tend to yield lower 2D:4D values compared with direct measurement from hand radiographs [29]. However, we used this measurement technique in all the participants. This would tend to result in non-differential misclassification of 2D:4D, which is not related to the outcome. Including participants whose fingers had features that might have affected the validity of the measurements in the analysis would have resulted in non-differential misclassification of 2D:4D categories,

which may have underestimated the observed associations. Thus sensitivity analyses were performed by excluding these participants and the results were similar. Another limitation of our study is that not all participants attended the follow-up during 2003–9, thus we do not have 2D:4D measures available for every participant. While the 2D:4D measurements were made between 2003 and 2009, the follow-up for total joint replacement commenced in 2001 and ended in 2011. Thus the 2D:4D data are a mixture of prospective and retrospective collection. As there is evidence that 2D:4D is stable over time [6], we consider it appropriate to analyse these data prospectively. Defining OA based on total joint replacements identifies partial evidence of the true problem. Whether patients undergo total joint replacement as a treatment of OA may be influenced by a number of factors, such as access to health care, physician bias and patient-level factors, in addition to disease severity [30]. Thus we performed the analysis on an age scale, stratified by sex and adjusted for country of birth to counter this issue.

In conclusion, in a large cohort study of middle-aged and older people, lower 2D:4D is associated with an increased risk of severe knee OA requiring TKR. This is not observed for severe hip OA. Although these results may be explained in part by joint injuries associated with high-level physical activity in those with lower 2D:4D and the greater susceptibility of knee OA in response to injury than hip OA, they may also reflect hormonal influences on the growth of bone, cartilage and soft tissue, which warrants further investigation.

Rheumatology key messages

- Lower index-to-ring finger length ratio (2D:4D) is associated with an increased risk of severe knee OA.
- 2D:4D is not associated with the risk of severe hip OA.
- There may be different mechanism of disease for knee and hip OA.

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3.3 Summary findings from chapter 3

Lower E2 concentration is associated with an increased risk of severe knee OA; whereas, lower ASD concentration and higher SHBG concentration are associated with risk of severe hip OA in women. Likewise, lower 2D:4D is associated with an increased risk of knee OA but not with severe hip OA. The findings from both the studies suggest that knee and hip OA are susceptible to different risk factors. Prevention strategies should be joint specific for knee and hip OA.

Chapter 4: Metabolic and vascular factors and risk of knee and hip osteoarthritis

Components of MetS (central obesity, hypertension, dyslipidaemia and hyperglycaemia) (91) and vascular comorbidities are more prevalent among the OA patients (33, 108, 111, 112). Obesity is the central component of MetS. Obese people had higher risk of vascular disease i.e. coronary atherosclerosis (141, 142). Obesity is the most important risk factor for the development and progression of knee OA (29, 40, 41), and a weaker risk factor for hip OA (29, 41). However the association between other MetS and vascular components with OA is not well understood. As age and obesity are the common risk factors for MetS, vascular disease and OA, it is unclear whether these conditions simply coexist due to common risk factors or there is a causal relationship. Previous studies have not adjusted for obesity largely, which is the major driver of OA, while exploring the relationship between MetS and OA. Moreover, no previous study has examined the microcirculation which is the essential and integral organ for tissue perfusion (115, 143) in the pathogenesis of OA.

This chapter addresses questions related to the relationship between MetS, microcirculation and OA and whether the relationship is independent of obesity. It incorporates two manuscripts; one examining the association of individual MetS components and MetS as a whole with the incidence of joint replacement for severe knee and hip OA, and the other examining the relationship between retinal vasculature which is a unique way to assess the microcirculation, and the incidence of joint replacement for severe knee OA.

Declaration for Thesis Chapter 4

Declaration by candidate

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

Manuscript: Incidence of total knee and hip replacement for osteoarthritis in relation to the metabolic syndrome and its components: A prospective cohort study. Seminars in Arthritis and Rheumatism 2014 43(4):429-36.

Nature of contribution	**Extent of contribution (%)**
Data analysis and interpretation, manuscript development and preparation	70%

The following co-authors contributed to the work. Where co-authors are students at Monash University, the extent of their contribution in percentage terms is stated:

Name	**Nature of contribution**	**Extent of contribution (%) for student co-authors only**
Wang	Study design, data analysis and interpretation, manuscript development and preparation	
Cicuttini	Study design, and manuscript editing	
Simpson	Data analysis and interpretation, and manuscript editing	
Giles	Study design, data collection, and manuscript editing	
Graves	Data acquisition and manuscript editing	
Wluka	Study design, data analysis and interpretation, and manuscript editing	

The undersigned hereby certify that the above declaration correctly reflects the nature and extend o the candidate's and co-author' contributions to this work

Candidate's Signature		**Date**
Main Supervisor's Signature		**Date**

Declaration for Thesis Chapter 4

Declaration by candidate

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

Manuscript: Retinal arteriolar narrowing and incidence of knee replacement for osteoarthritis: a prospective cohort study. (Under revision: Osteoarthritis Cartilage)

Nature of contribution	**Extent of contribution (%)**
Study design, data analysis and interpretation, manuscript development and preparation	75%

The following co-authors contributed to the work. Where co-authors are students at Monash University, the extent of their contribution in percentage terms is stated:

Name	**Nature of contribution**	**Extent of contribution (%) for student co-authors only**
Wang	Study design, data analysis and interpretation, and manuscript editing	
Shaw	Study design, data collection, manuscript editing	
Magliano	Data collection and manuscript editing	
Wong	Data collection and manuscript editing	
Wluka	Data interpretation, and manuscript editing	
Graves	Data acquisition and manuscript editing	
Tapp	Data collection and manuscript editing	
Cicuttini	Study design, data analysis and interpretation, and manuscript editing	

The undersigned hereby certify that the above declaration correctly reflects the nature and extend o the candidate's and co-author' contributions to this work

Candidate's Signature		**Date**
Main Supervisor's Signature		**Date**

4.1 Metabolic syndrome and risk of knee and hip osteoarthritis

Increasing evidence suggest an association between MetS and the risk of knee OA. The 3 year follow up of the ROAD study showed that the cumulative incidence of radiographic knee OA was 3.3% per year, and progression of knee OA for either knee was 8.0% per year (34). The odds ratio (OR) for the occurrence of knee OA was significantly increased according to the number of MetS components present (one component, 2.33; two components, 2.82; ≥three components, 9.83) (34). Similarly, progression of knee OA was significantly increased according to the number of MetS components present (34). The Malmö Diet and Cancer study reported the relationship between MetS and the incidence of severe knee OA was no longer significant after adjusting for BMI (RR: 1.1, 95% CI: 0.7-1.8), and MetS was not significantly associated with the incidence of hip OA (31).

Given the inconsistency of data examining components of MetS and OA, and whether MetS has the same effect on knee and hip OA, a longitudinal study was performed to explore the relationships between MetS and knee and hip joint replacement for OA in a community based population.

Hussain SM, Wang Y, Cicuttini FM, Simpson JA, Giles GG, Graves S, Wluka, AE. Incidence of total knee and hip replacement for osteoarthritis in relation to the metabolic syndrome and its components: A prospective cohort study. Semin Arthritis Rheum 2014; 43(4):429-36.

In this study, it was found that cumulative number of MetS components, central obesity and hypertension were associated with increased risk of severe knee OA and this association was independent of obesity measured by BMI. No associations were observed for severe hip OA.

These findings suggest that the pathogenesis of knee and hip OA differ and that targeting the management of MetS may reduce the risk of knee OA.

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Incidence of total knee and hip replacement for osteoarthritis in relation to the metabolic syndrome and its components: A prospective cohort study

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ABSTRACT

Objective: To examine whether components of metabolic syndrome (MetS), either singly or additively, were associated with the incidence of severe knee and hip OA, and whether these associations were independent of obesity assessed by body mass index (BMI).
Methods: Twenty thousand, four hundred and thirty participants who had blood lipids, anthropometric and blood pressure measurements during 2003–2007 were selected from the Melbourne Collaborative Cohort Study. MetS was defined as central obesity assessed by waist circumference and any two of raised triglyceride level, reduced HDL cholesterol level, hypertension or impaired fasting glycaemia. The incidence of total knee and hip replacement was determined by linking cohort records to the Australian Orthopaedic Association National Joint Replacement Registry.
Results: Six hundred and sixty participants had knee OA and 562 had hip OA. After adjustment for age, gender, country of birth, education, physical activity and BMI, central obesity [hazard ratio (HR) 1.59, 95% confidence interval (CI) 1.25–2.01] and hypertension (1.24, 1.05–1.48) were associated with increased risk of knee OA. The accumulation of MetS components was associated with knee OA risk, independent of BMI: one component, 2.12 (1.15–3.91); two components, 2.92 (1.60–5.33) and three or more components, 3.09 (1.68–5.69). No statistically significant associations were observed for hip OA.
Conclusion: Cumulative number of MetS components and central obesity and hypertension were associated with increased risk of severe knee OA, independent of BMI. No associations were observed with severe hip OA. These findings suggest that the pathogenesis of knee and hip OA differ and that targeting the management of MetS may reduce the risk of knee OA.

Introduction

Osteoarthritis (OA) is a major health problem with significant morbidity and disability associated with OA of the knees and hips. OA resulted in a total of 71.1 million years lived with disability in 2010, an increase of 64% since 1990 [1]. Although several drugs and nutriceuticals have been evaluated in clinical trials to determine whether they are able to slow the structural progression of OA, currently there are no registered disease-modifying OA drugs. Therefore, understanding the role of modifiable risk factors is important for improving prevention. Obesity [high body mass index (BMI) or waist circumference] is widely recognised as the most important modifiable risk factor for knee OA [2,3], although the evidence for hip OA is less consistent [2,4]. The available evidence suggests that obesity increases the risk of OA through both increased loading [5] and metabolic mechanisms [6,7].

There has been an increasing interest in the relationship between OA and the metabolic syndrome (MetS), the clustering of abdominal obesity, dyslipidaemia (low high-density lipoprotein (HDL) and triglyceridaemia), hyperglycaemia and hypertension [8].

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MetS was found to be 5.26 times more common in those with OA compared with those with no OA, and the individual components of the MetS were also more prevalent in people with OA in the NHANES III survey [9]. However, the Malmö Diet and Cancer study reported only central obesity to be associated with increased risk of knee OA independent of BMI, and neither MetS nor components of MetS to be associated with hip OA [10].

The Research on Osteoarthritis/Osteoporosis Against Disability (ROAD) 3-year follow-up study focused on the relationship between components of MetS defined as obesity measured by BMI, hypertension, dyslipidaemia and IFG with the incidence and progression of radiological knee OA in a Japanese population [11]. They reported that BMI, systolic blood pressure and dyslipidaemia were associated with incident knee OA [11], but the associations for systolic blood pressure and dyslipidaemia were no longer statistically significant after adjusting for BMI [11]. Moreover, this study found an increased risk of knee OA progression associated with an increase in the number of MetS components [11].

There is now an increasing body of evidence from epidemiological, proteomic, genetic and *in vitro* studies examining potential shared pathogenic mechanisms between the MetS and knee OA [12,13] that supports the inclusion of OA as a component of MetS [13], but it is still unclear whether MetS is a pathway to OA or whether OA and MetS simply coexist through commonly shared risk factors of age and obesity.

One method for defining OA is based on joint replacement [4,10]. This definition signifies severe clinical knee and hip OA, which is relevant to the symptomatic disease burden and economic impact of OA. Thus, in a large prospective cohort study, we examined the relationship between the components of MetS and the accumulation of components of MetS and the incidence of total knee and hip replacement due to severe OA.

Patients and methods

Study participants

The Melbourne Collaborative Cohort Study (MCCS) is a prospective cohort study of 41,514 participants (24,469 women) between the ages of 27 and 75 years at baseline [14]. Participants were recruited via the Electoral Rolls, advertisements and community announcements in local media, between 1990 and 1994. Southern European migrants to Australia (including 5411 Italians and 4525 Greeks) were deliberately oversampled to extend the range of lifestyle exposures and to increase genetic variation. The study protocol was approved by The Cancer Council Victoria's Human Research Ethics Committee. Follow-up was conducted by record linkage to Electoral Rolls, electronic phone books and the Victorian Cancer Registry and death records.

From 2003 to 2007, 28,046 participants (68% of the original MCCS participants) were followed up with face-to-face interviews. Of these, 20,430 had data available for all anthropometric measurements, blood pressure and blood lipids (Fig. 1), and thus were included for data analysis.

Anthropometric measurements

Height, weight, waist circumference and blood pressure (BP) were measured at the 2003–2007 follow-up visit according to written protocols based on standard procedures [15]. Weight was measured to the nearest 0.1 kg using digital electronic scales, height and waist circumference were measured to the nearest 1 mm using a stadiometer and a metal anthropometric tape, respectively. BMI was calculated as weight in kilograms divided by the square of height in metres. For BP recording, three

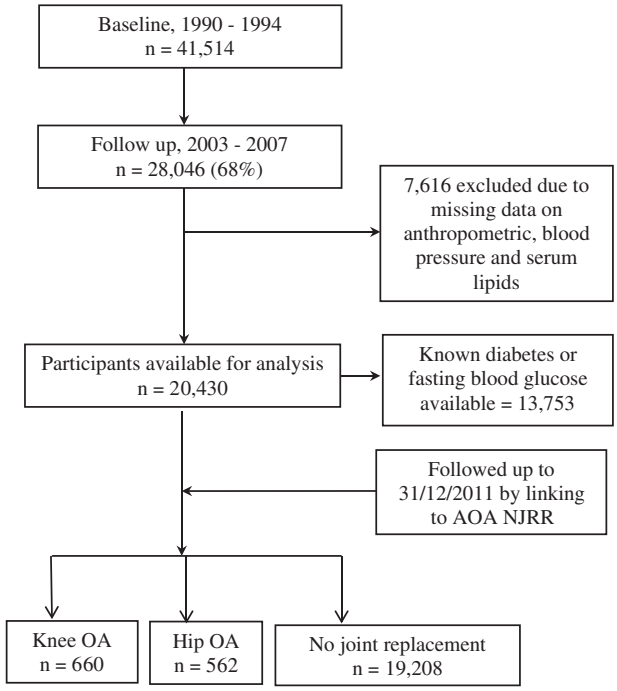

Fig. 1. Flowchart of data collection.

consecutive measurements were taken using a Dinamap 1846SX automatic BP monitor (Critikon, Tampa, FL). The average of the second and third BP measurements was used in the analysis.

Assessment of demographic, lifestyle factors and physical activity

At baseline, information was obtained regarding demographic and lifestyle factors, including date of birth, country of birth and highest level of education. Physical activity over the last 6 months was assessed by asking specific questions regarding the frequency of vigorous activity, less vigorous activity and walking. Walking and less vigorous activity frequencies were added together with twice the frequency of vigorous activity to compute a total physical activity level for each person. Physical activity was then categorised into four groups—none (0), low (> 0–4), moderate (> 4–6) and high (> 6) [16].

Plasma assays

Plasma lipid and glucose levels were measured by routine assays. Levels of serum lipids (triglyceride and HDL cholesterol) were measured using blood collected between 2003 and 2007. Total TG was measured enzymatically using a Hitachi 917 instrument (Boehringer Mannheim Corp., Indianapolis, IN). HDL cholesterol was measured using the HDL-C plus 2nd generation, Roche kit (Roche Diagnostics Australia Pty Ltd., Castle Hill, New South Wales, Australia) on a Hitachi 917 instrument (Boehringer Mannheim Corp, Indianapolis, IN) [17]. Since most of the participants were not fasting during 2003–2007 follow-up, plasma glucose levels measured from fasting blood collected at baseline (1990–1994) using a Kodak Ektachem analyzer (Rochester, NY) were the glucose levels used in the analysis.

Identification of incident primary knee and hip joint replacement

Cases were identified from the AOA NJRR. The Registry began data collection in September 1999 and implementation was introduced in a staged fashion in each of the Australian States and Territories. Victoria commenced data collection in 2001 [18]. The Registry monitors hip and knee joint replacements. It has

detailed information on the joint replacement prostheses, patient demographics, the reason for joint replacement, whether it is a primary joint replacement or a revision, and the type of revision. Data were collected from both public and private hospitals and were validated using a sequential multi-level matching process against State and Territory Health Department unit record data. Following the validation process and retrieval of unreported records, the Registry collects an almost complete and accurate set of data relating to hip and knee replacement in Australia [19].

Identifying information for MCCS participants, including first name, last name, date of birth and gender, was provided to the staff at the AOA NJRR in order to identify participants who had had a primary joint replacement performed between the time when they had fasting blood collected during 2003–2007 and December 31, 2011. The matching was performed on these data provided using the U.S. Bureau of the Census Record Linkage Software. Exact matches were identified and probabilistic matches were reviewed. The staff from the AOA NJRR forwarded this information to MCCS and it was then added to the MCCS database. The data linkage of the AOA NJRR with the MCCS was approved by The Cancer Council Victoria's Human Research Ethics Committee and the Monash University Human Research Ethics Committee.

Definition of the metabolic syndrome

For defining MetS, we used the definition of International Diabetes Federation. According to this definition, a person is defined to have MetS if he/she has central obesity (waist circumference $\geq$ 94 cm for men and $\geq$ 80 cm for women) and any two of the following four factors: raised serum triglyceride level ($\geq$ 1.7 mmol/L), reduced serum HDL cholesterol level ($<$ 1.03 mmol/L for males and $<$ 1.29 mmol/L for females, or specific treatment for these lipid abnormalities), raised blood pressure (systolic blood pressure $\geq$ 130 mmHg or diastolic blood pressure $\geq$ 85 mmHg, or treatment of previously diagnosed hypertension) and impaired fasting glycaemia (IFG) (fasting plasma glucose $\geq$ 5.6 mmol/L or previously diagnosed type 2 diabetes) [8].

Statistical analysis

Student's *t* test and chi square tests were used to compare the distribution of general characteristics (age, sex, BMI, country of birth, level of education and level of physical activity) between the attendees and non-attendees of the 2003–2007 follow-up, and also to determine whether the distribution of age, sex, BMI and the MetS components were similar for those with and without fasting blood glucose measurements available at baseline. Fasting blood sugar levels at follow-up and baseline were compared for the subgroup of participants whose fasting blood sugar was measured at both time points using the paired *t* test.

Cox proportional hazards regression models were used to estimate the hazard ratios (HRs) and 95% confidence intervals (CI) for first recorded primary total knee or hip joint replacement due to OA associated with each component of MetS, MetS itself and accumulation of MetS components. Follow-up for primary total joint replacement (i.e., calculation of person-time) began at the MCCS follow-up date between 2003 and 2007 when blood lipids, blood pressure and waist circumference were measured, and ended at the date of first primary total joint replacement for OA or date of censoring. Participants were censored at either the date of first primary joint replacement performed for indications other than OA, the date of death, the date they left Australia or end of follow-up (i.e., December 31, 2011, the date that ascertainment of joint replacement by NJRR was complete), whichever came first.

Linear associations between accumulation of MetS components and the risk of total joint replacement were investigated by comparing regression models with accumulation of MetS components as a categorical variable (0, 1, 2 and 3+ components) and a pseudo-continuous variable using the likelihood ratio test.

To assess whether associations between each component of MetS and MetS itself and the risk of total joint replacement was modified by sex or country of birth, interaction terms between sex or country of birth and each MetS component and MetS variable were fitted, and models with and without these interaction terms were compared using the likelihood ratio test.

All analyses were adjusted for age, sex, country of birth, education and physical activity. To examine whether the associations were confounded by total obesity (assessed by BMI), BMI was further adjusted.

Based on our sample size of 13,753 MCCS participants with complete data available on the MetS and joint replacement, we have 80% power (at the 5% significance level) to detect a risk ratio of 1.38, where the risk of total knee replacement or total hip replacement in those without MetS was assumed to be 2.5% and the prevalence of MetS to be 28%.

Tests based on Cox regression methods showed no evidence that proportional hazard assumptions were violated for any analysis. All statistical analyses were performed using Stata 12.0 (Stata Corp LP., College Station, TX).

Results

A total of 1222 total joint replacements (660 total knee replacements and 562 total hip replacements) performed for OA were identified between the 2003 and 2007 follow-ups and December 31, 2011. The mean follow-up duration was 6.8 (SD 1.5) years. There were differences in relation to the distribution of age, sex, BMI, waist circumference, prevalence of hypertension, impaired fasting glucose, country of birth, education and physical activity between the attendees and non-attendees of the 2003–2007 follow-up, which showed that the non-attendees had worse health condition (Supplementary Table 1). Further, for the small percentage of participants (n = 735; 2.6% of 2003–2007 sample) whose fasting blood glucose was measured during the second follow-up, the follow-up and baseline fasting blood glucose levels were very similar (mean difference 0.05 mmol/L; 95% CI 0.04–0.07). The distribution of the MetS components at the 2003–2007 follow-up were similar for those with (n = 13,753) and without (n = 6677) fasting blood glucose measurements at baseline (Supplementary Table 2).

Descriptive statistics for selected characteristics of the study participants are presented in Table 1. Participants who received a total joint replacement for OA were older, and participants undergoing a total knee replacement were heavier and had greater BMI and waist circumference than those with no joint replacement. Most of the components of MetS and the MetS were more prevalent for participants who underwent total knee or hip joint replacement for severe OA. These results were more evident for those who had a total knee replacement. Further, participants born in Italy or Greece were less likely to require a total joint replacement compared with those from Australia or UK origin.

Each component of MetS and presence of MetS was significantly associated with increased risk of severe knee OA after adjustment for age, sex, country of birth, education and physical activity (Table 2, Model 1). After including BMI in the models, the associations of central obesity (HR 1.59, 95% CI 1.25–2.01) and hypertension (HR 1.24, 95% CI 1.05–1.48) remained statistically significant (Table 2, Model 2).

Relationships between accumulation of factors of MetS and the risk of severe knee OA are presented in Figure 2. With the

Table 1
Characteristics of the study participants

	Primary knee replacement (n = 660)	Primary hip replacement (n = 562)	No joint replacement (n = 19,208)
Age at 2003–2007 (years)	68.5 ± 7.6	68.0 ± 7.9	64.8 ± 8.6
Age at joint replacement (years)	72.2 ± 7.6	71.6 ± 7.9	
Women, n (%)	427 (64.6)	382 (67.8)	11,427 (59.3)
Body mass index (kg/m^2)	29.7 ± 5.3	27.3 ± 4.7	26.8 ± 4.5
Country of birth, n (%)			
Australia	564 (85.3)	489 (86.9)	15,248 (79.2)
United Kingdom	50 (7.6)	50 (8.9)	1536 (8.0)
Italy	37 (5.6)	20 (3.5)	1697 (8.8)
Greece	10 (1.5)	4 (0.7)	780 (4.0)
Education level, n (%)			
Primary and some high school	360 (54.6)	270 (47.9)	8868 (46.2)
Completed high school and degree/diploma	299 (45.5)	239 (52.1)	10,353 (53.9)
Total physical activity level, n (%)			
None (0)	119 (18.0)	101 (17.9)	3693 (19.2)
Low (0–4)	124 (18.8)	107 (19.0)	3840 (19.9)
Moderate (4–6)	239 (36.2)	179 (31.8)	6633 (34.4)
High (> 6)	179 (27.1)	176 (31.3)	5094 (26.4)
Central obesity, n (%)	555 (84.0)	383 (68.0)	12,023 (62.4)
Low HDL, n (%)	87 (13.2)	62 (11.0)	2333 (12.1)
Triglyceridaemia, n (%)	170 (25.7)	103 (18.3)	4016 (20.8)
Hypertension, n (%)	457 (69.1)	336 (59.7)	10,836 (56.3)
IFG,[a] n (%)	199 (43.1)	143 (39.1)	4498 (34.8)
MetS,[a] n (%)	198 (42.9)	117 (32.0)	3635 (28.1)
No. of components of MetS[a]			
0 comp (n = 1687)	12 (2.6)	27 (7.4)	1648 (12.7)
1 comp (n = 3577)	78 (16.9)	94 (25.7)	3405 (26.3)
2 comp (n = 4198)	162 (35.1)	124 (33.9)	3912 (30.3)
≥ 3 comp (n = 4291)	210 (45.4)	121 (33.1)	3960 (30.6)

HDL, high-density lipoprotein; IFG, impaired fasting glycaemia; MetS, the metabolic syndrome.
[a] Data available for 13,753 participants.

accumulation of MetS components, the risk of total knee replacement due to severe OA rose. This association was attenuated but still positive and of public health significance after accounting for BMI. The HRs for cumulative MetS components after adjustment for age, sex, country of birth, education and physical activity and BMI are as follows: one component, 2.12 (95% CI 1.15–3.91, p = 0.02); two components, 2.92 (95% CI 1.60–5.33, $p < 0.001$) and three or more components, 3.09 (95% CI 1.68–5.69, $p < 0.001$). However, the association was not linear (p for likelihood ratio test = 0.04).

The relationships between individual components and the presence of the MetS and hip joint replacement for OA were also examined (Table 3). Only central obesity was associated with increased risk of severe hip OA in Model 1. However, when BMI was introduced in Model 2, the association of central obesity was no longer statistically significant. There was no significant association between the cumulative MetS components and the risk of severe hip OA. The HRs for cumulative MetS components after adjustment for age, sex, country of birth, education and physical activity and BMI are as follows: one component, 1.29 (95% CI 0.84–2.00, p = 0.25); two components, 1.28 (95% CI 0.82–1.99, p = 0.28) and three or more components, 1.17 (95% CI 0.74–1.87, p = 0.50) (Fig. 3).

Tests for interaction were performed to determine if sex and country of birth modified the association between the components of MetS and the risk of knee or hip OA. No effect modification was observed (all p values for likelihood ratio test > 0.10).

Discussion

In a large prospective cohort study, we found that the MetS and its components, central obesity, hypertension, low HDL, triglyceridaemia and IFG were associated with increased incidence of severe knee OA requiring total knee replacement. Following adjustment for BMI, a measure of overall body size, the associations of central obesity, hypertension and the MetS remained significant. With the accumulation of MetS components, the incidence of severe knee OA increased, independent of BMI.

Table 2
Incidence of total knee replacement due to osteoarthritis in relation to components of the metabolic syndrome

	Model 1		Model 2	
	Hazard ratio (95% CI)	p Value	Hazard ratio (95% CI)	p Value
Central obesity	3.06 (2.48–3.77)	< 0.001	1.59 (1.25–2.01)	< 0.001
Low HDL	1.13 (0.90–1.42)	0.28	0.80 (0.63–1.01)	0.06
Triglyceridaemia	1.34 (1.13–1.60)	0.001	0.99 (0.83–1.18)	0.91
Hypertension	1.35 (1.14–1.61)	0.001	1.24 (1.05–1.48)	0.01
Impaired fasting glycaemia[a]	1.36 (1.12–1.64)	0.002	1.07 (0.88–1.30)	0.46
MetS[a]	1.92 (1.59–2.32)	< 0.001	1.24 (1.02–1.52)	0.03

CI, confidence interval.
Model 1: adjusted for age, gender, country of birth, level of education and physical activity.
Model 2: adjusted for age, gender, country of birth, level of education, physical activity and BMI.
[a] Data available for 13,753 participants.

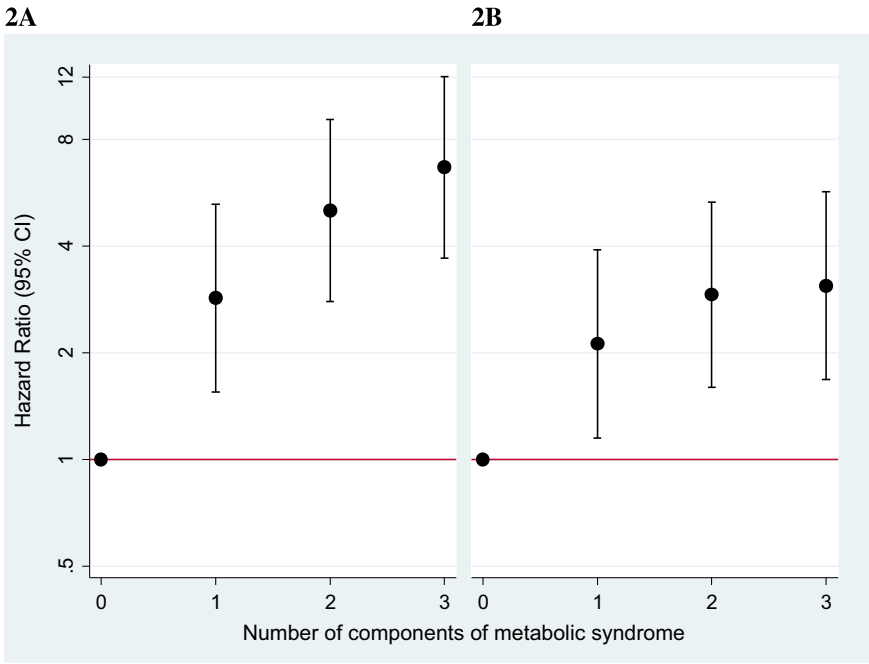

Fig. 2. (A) Hazard ratios of total knee replacement for OA related to the accumulation of components of the metabolic syndrome, adjusted for age, gender, country of birth, physical activity and level of education (n = 13,753). (B) Hazard ratios of total knee replacement for OA related to the accumulation of components of the metabolic syndrome, adjusted for age, gender, country of birth, physical activity, level of education and BMI (n = 13,753).

In contrast, the incidence of severe hip OA was not related to either the individual or cumulative MetS components.

We have confirmed central obesity as a strong risk factor for the incidence of severe knee OA, independent of BMI. This finding is consistent with several other studies that have reported an association between knee OA and waist circumference [2,4,20] and adipose tissue mass [2,4].

We found a positive association between hypertension and the incidence of severe knee OA, independent of BMI. Several epidemiological studies have shown that OA is more common among hypertensive patients [3,7,13]. Some studies that showed an association between hypertension and OA did not adjust for BMI [9,21], which is a strong determinant of hypertension [27]. In other studies that adjusted for BMI, the association between hypertension and OA was no longer significant after the adjustment [10,11]. In contrast, we found that the relationship between hypertension and severe knee OA persisted after adjusting for BMI, suggesting that this relationship is independent of overall obesity.

Several epidemiological studies have shown that elevated serum cholesterol is a risk factor for knee OA [3,22,23]. This was further strengthened by the results of the Chingford study that showed that this relationship persisted after adjusting for obesity assessed by BMI [3]. Additionally, Davies-Tuck et al. [24] showed increased level of serum cholesterol and triglyceride to be associated with bone marrow lesions (BMLs) at the knee. Likewise, Doré et al. [25] showed a weak association with low HDL and BMLs. In contrast, the Malmö study found that low HDL and triglyceridaemia had no association with incidence of knee OA [10]. The ROAD study also could not establish any association independent of obesity between low HDL and the progression of knee OA [11]. Whilst we found that triglyceridaemia was associated with the incidence of severe knee OA, this association was not independent of BMI. Additionally, we found no association between low HDL and knee OA. In our study, hip OA was associated with neither low HDL nor triglyceridaemia, which is consistent with the finding of the Malmö study [10]. Differences in body composition may account for these discrepancies, as increased fat mass is associated with increased dyslipidaemia [26]; none of these studies accounted for body composition.

IFG has been found to be associated with adverse structural changes (increased cartilage volume loss and incident BMLs) over 2 years in the knee for healthy women without knee OA or diabetes [27]. Cross-sectional studies showed conflicting findings for the association between blood glucose levels and OA, as some

Table 3
Incidence of total hip replacement due to osteoarthritis in relation to components of the metabolic syndrome

	Model 1		Model 2	
	Hazard ratio (95% CI)	p Value	Hazard ratio (95% CI)	p Value
Central obesity	1.30 (1.08–1.55)	0.004	1.04 (0.83–1.30)	0.72
Low HDL	0.96 (0.74–1.25)	0.78	0.86 (0.66–1.13)	0.28
Triglyceridaemia	0.91 (0.73–1.13)	0.40	0.81 (0.65–1.01)	0.07
Hypertension	0.92 (0.77–1.10)	0.37	0.89 (0.75–1.07)	0.22
Impaired fasting glycaemia[a]	1.19 (0.96–1.48)	0.11	1.10 (0.88–1.37)	0.39
MetS[a]	1.19 (0.95–1.49)	0.12	1.00 (0.78–1.27)	0.98

CI, confidence interval.
Model 1: adjusted for age, gender, country of birth, level of education and physical activity.
Model 2: adjusted for age, gender, country of birth, level of education, physical activity and BMI.

[a] Data available for 13,753 participants.

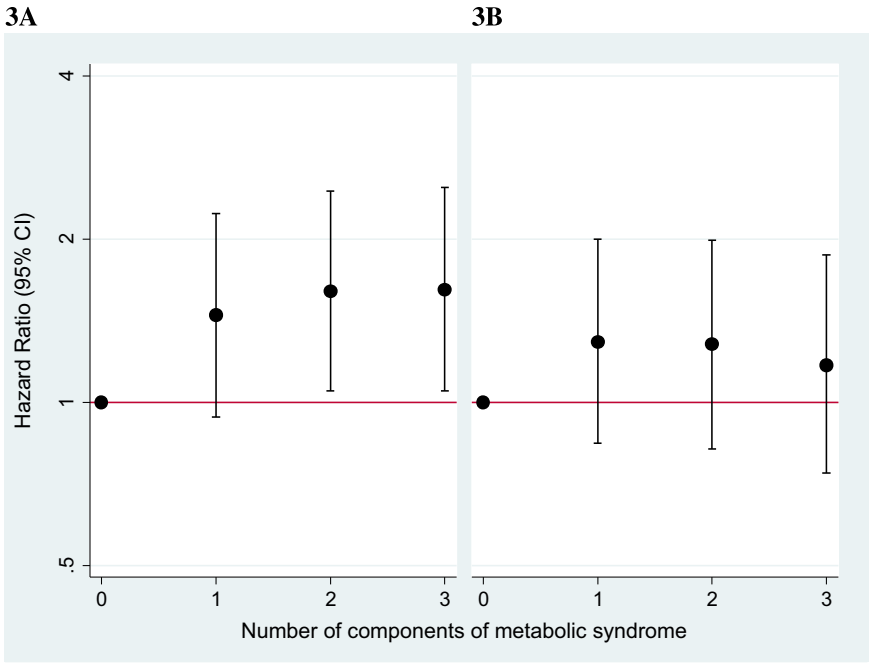

Fig. 3. (A) Hazard ratios of total hip replacement for OA related to the accumulation of components of the metabolic syndrome, adjusted for age, gender, country of birth, physical activity and level of education (n = 13,753). (B) Hazard ratios of total hip replacement for OA related to the accumulation of components of the metabolic syndrome, adjusted for age, gender, country of birth, physical activity, level of education and BMI (n = 13,753).

of the studies showed a positive association [6,28,29], while the others found no association [20,30]. Results from two cohort studies in relation to hyperglycaemia were also inconsistent. Engström et al. [10] showed that the presence of hyperglycaemia was associated with increased risk of knee OA (RR 2.2, 95% CI 1.4–3.6), but after adjustment for BMI, this finding was no longer significant—the study probably did not have the power to detect the identified risk ratio (RR 1.4, 95% CI 0.9–2.4). In contrast, a cohort study of 927 participants over 20 years reported diabetes to be associated with increased risk of hip or knee OA (HR 2.1, 95% CI 1.1–3.8), after adjustment for 12 confounders including BMI [31]. But this analysis was based on 13 replacements among 69 diabetes patients and did not examine hip and knee OA separately [31]. Consistent with the Malmö study, our study found that IFG was associated with the incidence of knee OA but not independent of BMI, with no association seen for hip OA. Different studies have used different cut-off points for defining hyperglycaemia, some of them have used diabetes instead of hyperglycaemia [9,31,32], measured HbA1c [11,33], others depended on self-reported diabetes [20], which is likely to miss 50% of those with diabetes [34]. We used both plasma glucose or previously diagnosed for IFG measurement. These may explain the variation in the findings.

We found the accumulation of components of MetS to be associated with the incidence of severe knee OA as assessed by knee replacement, independent of BMI. The ROAD study, which examined radiological knee OA, reported similar findings, but they were not adjusted for obesity [11,35]. Our results suggest that, although the relationship between some of the individual components of MetS and knee OA might be weak, the cumulative MetS components increase the risk of severe knee OA.

It is likely that the mechanism by which MetS influences OA is complex. The homoeostasis between adipogenesis and osteogenesis and/or chondrogenesis is probably modified in people with OA [36]. In early stages of OA development, substantial stores of lipid deposits have been noted in chondrocytes [37]. Also, those with MetS have an adverse metabolic profile including dyslipidaemia [22,23], an abnormal metabolic environment, with the presence of adipocytokines [38] and the presence of low-grade inflammation [39,40], hyperglycaemia [5], insulin resistance [41] and hypertension [3,42], all of which may contribute to the initiation and progression of OA [12]. It may also be that a common pathway is via vascular pathology and affects joint nutrition [43]. The effect of MetS on blood vessels over time may result in vessel narrowing [21,43], reduced blood flow [43] and subchondral ischaemia, leading to cartilage degeneration due to reduced nutrient and gas supply from the subchondral bone [43,44]. This may also trigger apoptosis of osteocytes and activate osteoclastic resorption and reduced bony support for the overlying cartilage [45]. Total joint replacement is indicated for people with severe pain in the presence of joint damage. There is evidence for a role of inflammatory mediators on nociception and pain in arthritis [46]. It is possible that people with MetS, which is accompanied by meta-inflammation, have higher levels of pain for the same severity of structural OA, which makes them more likely to decide to undergo a total joint replacement.

Although we found a relationship between the MetS and its components and severe knee OA, no relationship was observed for severe hip OA. The findings are consistent with those from the Malmö study [10]. The differed finding for knee and hip OA in relation to MetS may be due to different susceptibility of these two joints to metabolic factors [47]. The knee may be more dependent on soft tissue and neuromuscular control for its stability. Metabolic factors have been shown to affect soft tissue integrity. For example, MetS has been associated with intracellular fat deposition in muscle [48] and tendon structure [48,49]. In contrast, at the hip, bony shape and joint congruence appear to have a greater role in the development of hip OA [50], making the hip less susceptible to the effects of meta-inflammation. Our findings provide further support for the available evidence that differences exist between the pathogenesis of knee and hip OA.

Our study has several strengths and limitations. The large size of the cohort with complete follow-up of total joint replacement, the prospective design and inclusion of participants from different ethnic backgrounds are the major strengths. Additionally, we have examined the relationship of MetS and OA of knee and hip in the same population, and have demonstrated that the mechanism of disease differs at these joints. Total joint replacements tend to be

offered to patients who are otherwise relatively medically fit. The reluctance of orthopaedic surgeons to operate on patients who are obese or with obesity-related comorbidities would have resulted in an underestimation of the associations of the MetS and its components with the risk of total joint replacement for OA observed in our study. Approximately, 68% of the original MCCS participants had serum lipid measurement and were included in the analysis. Fasting serum glucose results were not available for all participants at the same time when the serum lipids were measured, thus we used serum glucose at study inception. However, we compared the difference between fasting serum glucose levels at baseline and when lipid levels were taken (for those for whom serum glucose was measured at both time points) and found no significant difference in serum glucose. The use of lipid lowering and hypoglycaemic medications was not recorded. This may have resulted in participants with naturally adverse serum lipid and glucose profiles being classified as having normal serum measures. This may partially explain the marginal negative association between triglyceridaemia and the incidence of total hip joint replacement. Moreover, this is likely to be non-differential in relation to the outcomes (incidence of knee and hip OA) and thus biased our results towards the null. Nevertheless, we showed significant results at the knee but not the hip.

Conclusion

The MetS itself and individual components of the MetS, particularly central obesity and hypertension, are associated with increased incidence of severe knee OA requiring total knee replacement, independent of obesity assessed using BMI. Furthermore, accumulation of components of the MetS in an individual increases the risk of severe knee OA, independent of BMI. No such relationship was seen for severe hip OA. Our findings suggest that the pathophysiology of knee and hip OA differ and that management of the MetS may reduce the burden of knee OA.

Acknowledgements

The Melbourne Collaborative Cohort Study was made possible by the contribution of many people, including the original investigators and the diligent team who recruited the participants and who continue working on the follow-up. We would like to express our gratitude to the many thousands of Melbourne residents who continue to participate in the study. For the data linkage, we would especially like to thank the Registry coordinator Ann Tomkins and statistician Lisa Miller from the Australian Orthopaedic Association National Joint Replacement Registry, and Ms Georgina Marr from Cancer Council Victoria.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.semarthrit.2013.07.013.

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4.2 Vascular factors and risk of knee osteoarthritis

Although the signature feature of OA is articular cartilage loss, recent evidence suggests that OA is a disease that affects the whole joint (3, 4). Articular cartilage itself is avascular, and depends on the surrounding synovial fluid and the underlying subchondral bone for receiving its oxygen and nutrition (144, 145). The exchange of nutrients between blood and tissue occurs almost exclusively through the microcirculation and the earliest manifestation of vascular disease occurs in the micro circulatory bed (143). Though, recent research has linked knee OA with large vessel atherosclerosis, for example, carotid intima media thickness (106) and popliteal artery wall thickness (117), there is a paucity of research examining the microcirculation and the risk of knee OA.

This longitudinal study examined the association between microcirculation measured by retinal vascular calibre and the incidence of knee replacement for OA.

Hussain SM, Wang Y, Shaw JE, Magliano DJ, Wong TY, Wluka AE, Graves S, Tapp RJ, Cicuttini FM. Retinal arteriolar narrowing and incidence of knee replacement for osteoarthritis: a prospective cohort study. (Under Revision)

This study demonstrated an association between retinal arteriolar narrowing and increased risk of knee replacement for OA. This result suggests a role of microcirculation in the pathogenesis of knee OA. Although more work is needed to confirm the association, it can be assumed that, modification of microcirculation via lifestyle and pharmacological interventions may represent a potential target for the prevention and treatment of knee OA.

Retinal arteriolar narrowing and incidence of knee replacement for osteoarthritis: a prospective cohort study

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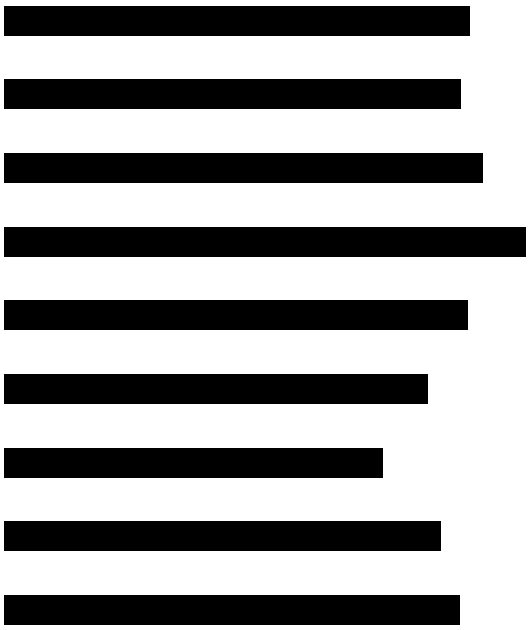

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Running title: Retinal arteriolar narrowing and osteoarthritis

Abstract

Objectives: The role of the microcirculation in the pathogenesis of osteoarthritis remains unclear. This prospective cohort study examined the association between retinal vascular calibre and incidence of knee replacement for osteoarthritis.

Design: 1,838 participants of the Australian Diabetes, Obesity and Lifestyle Study had retinal vascular calibre measured using a nonmydriatic digital fundus camera in 1999-2000 and were aged ≥40 years at joint replacement data collection commencement. The incidence of knee replacement for osteoarthritis during 2002-2011 was determined by linking cohort records to the Australian Orthopaedic Association National Joint Replacement Registry.

Results: 77 participants underwent knee replacement for osteoarthritis. They had narrower retinal arteriolar calibre compared with those without knee replacement (166.1±24.8μm vs. 174.3±24.5μm, p=0.004). For every 1 standard deviation reduction in retinal arteriolar calibre, the incidence of knee replacement increased by 25% (HR 1.25, 95%CI 1.00-1.56). Participants in the narrower two-thirds of arteriolar calibre had twice the risk of knee replacement compared with those in the widest one-third (HR 2.00, 95%CI 1.07-3.74, p=0.03) after adjustment for sex, body mass index, physical activity and HbA1c. There was no association for retinal venular calibre.

Conclusions: Retinal arteriolar narrowing is associated with increased risk of knee replacement for osteoarthritis, suggesting a role of the microcirculation in the pathogenesis of knee osteoarthritis.

Key words: Osteoarthritis, knee replacement, microcirculation, retinal arteriolar calibre, retinal venular calibre

Introduction

There is increasing evidence suggesting an association between vascular disease and osteoarthritis (OA)[1, 2]. The Rotterdam study found that increased intima media thickness of the carotid artery, a subclinical marker of large vessel atherosclerosis, was associated with increased prevalence of knee OA and progression of hand OA in women[1]. In the women of the AGES Reykjavik Study, carotid plaque and coronary calcification were associated with increased severity of hand OA[2]. Increased popliteal artery wall thickness, another measure of large vessel atherosclerosis, was associated with generalized OA[3]. These findings suggest that macrovascular disease is involved in the pathogenesis of OA possibly through reduced nutrition to the joint. However, little work has examined the role of microcirculation in OA.

The microcirculation, including the arterioles, capillaries, and venules, optimizes nutrient and oxygen supply within tissues in response to metabolic demand variations[4]. Some of the earliest manifestation of cardiovascular disease occurs in the microcirculatory bed[4]. Abnormal microcirculation is common among the conventional cardiovascular risk factors, including hypertension, diabetes, obesity, and dyslipidemia[4] which are risk factors for OA[5]. The retinal vasculature provides a unique window to assess the microcirculation noninvasively and directly[6] and it has been associated with clinical and subclinical cardio-metabolic outcomes including hypertension[7, 8], dyslipidaemia[7], and diabetes[8]. The purpose of this study was to examine the association between retinal vascular calibre and the incidence of knee replacement for OA.

Patients and Methods

The Australian Diabetes, Obesity and Lifestyle (AusDiab) Study is a population-based, national prospective cohort study of 11,247 people, aged ≥25 years, recruited during 1999-

2000[9]. 2,476 participants (having diabetes or impaired glucose metabolism, and a random sample with normal glucose tolerance) who participated in the baseline complications survey had retinal vascular calibre measured and were included in this study[10]. They were older (60.5±12.0 vs. 54.8±11.9 years), had higher HbA1c level (5.7±1.2 vs. 5.2±0.5), systolic blood pressure (138.9±20.1 vs. 130.0±18.6 mmHg), and BMI (28.7±5.6 vs. 26.8±4.7 kg/m^2) (all $p<0.001$) than non-participants. We restricted our analysis to those aged ≥40 years at joint replacement data collection commencement (n=1,838) since joint replacement as treatment of OA is very uncommon under this age.

Data on date of birth, sex, and physical activity (sufficient, ≥150 minutes per week, insufficient, <150 minutes per week, or sedentary, 0 minute per week) were collected by trained interviewers[9]. Height and weight were measured using standard protocols; systolic blood pressure measured with dinamap/mercury sphygmomanometer; HbA1c measured by Boronate affinity high performance liquid chromatography; serum total cholesterol measured by enzymatic method; and urine protein measured by immunoturbidimetric method (Olympus AU600 analyser)[9].

Retinal photographs of both eyes were taken using a nonmydriatic digital fundus camera[10]. Retinal vascular calibre was measured using a validated computer-based program[10]. For each photograph, the average arteriolar and venular calibre was summarized as central retinal artery equivalent and central retinal vein equivalent, respectively[10]. Reproducibility of this method was high, with intra- and inter-grader intra-class correlation coefficients 0.78–0.99[10].

The Australian Orthopaedic Association National Joint Replacement Registry (AOANJRR) collects information on prostheses, patient demographics, type and reason for joint

replacement, with almost complete data on joint replacement in Australia[11]. Linking AusDiab records to AOA NJRR identified those who had a primary joint replacement performed between 1 January 2002 and 31 December 2011. Knee OA was defined as the first primary knee replacement for OA. For those with multiple joint replacements, only the first recorded knee replacement was considered. Data linkage study was approved by the Alfred Hospital, University of Adelaide and Monash University Human Research Ethics Committees. All participants gave written informed consent.

Cox proportional hazard regression models were used to estimate the hazard ratio (HR) and 95% confidence interval (CI) for the incidence of knee replacement due to OA associated with retinal vascular calibre, with age as the time scale. Follow-up for joint replacement (calculation of person-time) began 1 January 2002, and ended at the date of first knee replacement for OA or date of censoring. Participants were censored at either the date of first knee replacement for indications other than OA, the date of death, or end of follow-up (December 31, 2011), whichever came first. Retinal vascular calibre was standardized so that HR represents the effect of a one-standard-deviation difference in calibre. Retinal vascular calibre was also categorized into tertiles based on the analysis sample with widest tertile used as the referent category. Linear association between retinal vascular calibre and knee replacement risk was examined using the likelihood ratio test. Each analysis was adjusted for sex and BMI, and further adjusted for physical activity, HbA1c, and cardiovascular risk factors (systolic blood pressure, total cholesterol and microalbuminuria). All statistical analyses were performed using Stata 12.0 (StataCorp LP., College Station, TX, USA).

Results

Over the 8.7 (SD 2.7) years of follow-up, 77 knee replacements for OA were identified. Characteristics of the study participants are presented in Table 1.

After adjusting for sex, BMI, HbA1c and physical activity, 1 standard deviation reduction in retinal arteriolar calibre was associated with a 25% increased incidence of knee replacement (HR 1.25, 95% CI 1.00-1.56) (Table 2). When retinal arteriolar calibre was examined as a categorical variable, HR was 1.98 (95% CI 1.00-3.92) for the narrowest and HR 2.02 (95% CI 1.03-3.94) for the middle tertile, with widest tertile the referent group. Further adjustment for the cardiovascular risk factors did not change the associations. As there was no evidence for a linear association, the narrower 2 tertiles were combined and compared with the widest tertile. Participants with narrower two-thirds of arteriolar calibre had a 2 times increased risk of knee replacement (HR 2.00, 95% CI 1.07-3.74, p=0.03) after adjustment for sex, BMI, physical activity and HbA1c. There was no association observed for retinal venular calibre.

Discussion

We found that narrower retinal arteriolar calibre, but not retinal venular calibre, predicted an increased risk of knee replacement for OA independent of age, sex, BMI, physical activity, HbA1c and other cardiovascular risk factors.

While previous studies have shown an association between macrocirculation and OA risk[1-3], only one cross-sectional study reported an association between microcirculation and early structural changes of OA[12]. A wider retinal venular calibre was associated with increased risk of knee bone marrow lesions (BML) in an asymptomatic population[12]. In contrast, we found an association of narrower retinal arteriolar calibre but not wider retinal venular calibre with

knee replacement risk. The discordant findings may be due to the different stages of OA process. The previous study of an asymptomatic population found that wider venular calibre, which is associated with inflammation, was associated with BML, a predictor of cartilage loss[13]. This current study found a relationship between smaller arteriolar calibre and increased incidence of knee replacement for OA, i.e. the symptomatic end-stage OA where significant cartilage loss has already occurred. These results suggest that microcirculation has a different role in healthy knee/early OA and late stage OA. Given the evidence that risk factors for OA progression are not the same as those for OA development, our findings support the notion that atheromatous vascular disease is important in the progression of OA[13]. Retinal arteriolar calibre is a measure assessing microcirculation with narrower arteriolar calibre associated with higher blood pressure[7, 8], obesity[7], dyslipidaemia[7], diabetes[8], and coronary heart disease[7, 8]. These cardio-metabolic abnormalities have been linked with knee OA[5]. Our study showed an association between narrower retinal arteriolar calibre and increased risk of knee replacement for OA independent of these cardio-metabolic factors.

While the mechanisms for the relationship between knee OA and narrower arteriolar calibre which reflects generalised microvascular pathology[6], are unknown, it may be due to impaired tissue perfusion in the synovium and subchondral bone. Articular cartilage is avascular, it depends on synovial fluid and subchondral bone for nutrition and for structural support[14]. The supply of glucose, oxygen and water requirements of articular cartilage are provided by perfusion from synovial fluid and subchondral vessels[14]. Synovium, a highly vascular tissue with arterioles, capillaries and venules, produces synovial fluid and is the main source of nutrition for articular cartilage[15]. Narrower arterioles in synovium result in localised hypoxia that stimulates angiogenesis, development of an immature vasculature and inflammation predictive of cartilage damage and catabolic effects on chondrocytes in OA[15]. Bone is a

highly vascular structure and its growth, repair and metabolism as well as modelling and remodelling highly depend on blood flow and haemopoiesis[14]. Ischaemic episodes in the subchondral bone lead to increased bone resorption and subsequent articular damage in OA[14]. Therefore it is likely that narrowing of arterioles impairs the integrity of subchondral bone and the supply of oxygen and nutrients to the overlying cartilage plate, resulting in subsequent cartilage damage[14]. Moreover, the knee depends on soft tissue and neuromuscular control for its stability. Arteriolar narrowing may also affect soft tissue integrity. There is evidence that arteriolar narrowing is associated with decreased perfusion of skeletal muscle, resulting in muscular atrophy[16]. Our findings support the notion that the microcirculation is involved in the pathogenesis of knee OA through arteriolar narrowing.

This study should be considered in context of its limitations. The AusDiab participants did not have knee x-rays. We used knee replacement for OA, rather than Kellgren Lawrence grade $\geq$2 to define knee OA. Some individuals without knee replacement may have had radiographic OA, resulting in misclassification of knee OA. However this is likely to be non-differential misclassification and underestimate the associations. Although joint replacement due to OA will only identify the tip of the iceberg of OA, it has the advantage of recognising severe OA which has an unambiguous connection with disease burden. Joint replacement is influenced by a number of factors such as access to health care, socioeconomic status, and preference of the patient, in addition to disease severity. This study was carried out in Australia where there is universal health cover, so access to joint replacement is available to all with knee structural change is a risk factor for knee replacement in this setting[17]. We have performed analysis with age as the time scale and adjusted for sex, BMI, physical activity and other metabolic factors. We did not have replacement data prior to 2002. This may have resulted in non-differential misclassification of joint replacement which is likely to bias the

results to the null. AusDiab data does not have measurement of middle-sized peripheral arteries, so we could not compare retinal vascular calibre with middle-sized artery atherosclerosis in relation to OA. We did not find a linear relationship between retinal arterial calibre and risk of knee replacement. However, a linear relationship is not an essential criterion for assessing causality and it depends on the mechanism of action of the risk factor[18]. There may be selection bias since our study population was enriched for those most likely to have diabetes. Therefore caution should be taken to generalize the findings to the general population. When stratified analysis was performed based on diabetic status, we found similar association between retinal arteriolar narrowing and risk of knee replacement for OA irrespective of diabetes (data not shown). Examination of knee replacement for OA was not the primary goal of the AusDiab study. The results must be taken in context of the whole body of evidence. The findings of this study add to the current knowledge regarding microvascular changes linked to the risk of knee OA after controlling for potential confounders. Although the findings of this study add to the current knowledge that altered microcirculation may play a role in the development and/or progression of knee OA, further research is needed to confirm the association. The strengths of our study include its prospective design, use of a validated computer software program to measure retinal vascular calibre, and the validation and completeness of AOA NJRR data[11].

This study showed an association between retinal arteriolar narrowing and increased risk of knee replacement for OA, suggesting a role of microcirculation in the pathogenesis of knee OA. Although more work is needed to confirm the association, modification of microcirculation via lifestyle and pharmacological interventions may represent a potential target for the prevention and treatment of knee OA.

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Author contributions

SMH, YW and FC were involved in conception and design of the study. SMH was involved in statistical analysis and interpretation of the data, and drafted the manuscript. YW was involved in cleaning and merging the datasets and interpretation of the data. AW was involved in the interpretation of the data. JES, DJM, TYW, RJT and SG were involved in the acquisition of the data. All authors reviewed the manuscript and approved the final manuscript. FC is the guarantors.

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Table 1: Characteristics of study population

	No knee replacement (n =1,761)	Knee replacement (n =77)	P value
Age at baseline (years)	60.3±12.1	65.0±7.5	0.001
Age at 1 January 2002 (years)	62.5±12.1	67.4±7.5	0.001
Female, n (%)	965 (54.8)	39 (50.7)	0.47
Body mass index (kg/m^2)	28.6±5.6	31.4±5.5	<0.001
Physical activity, n (%)			0.20
Sedentary	376 (21.5)	23 (29.9)	
Insufficient	572 (32.7)	24 (31.2)	
Sufficient	803 (45.9)	30 (39.0)	
HbA1c (%)	5.7±1.2	5.8±1.1	0.35
Systolic blood pressure (mmHg)	138.4±20.2	149.0±18.3	<0.001
Total cholesterol (mmol/L)	5.7±1.0	5.7±1.0	0.63
Microalbumin (mg/L)	33.5±118.0	20.1±55.6	0.34
Retinal arteriolar calibre (µm)	174.3±24.5	166.1±24.8	0.004
Retinal venular calibre (µm)	205.0±23.2	198.4±21.6	0.01

Data presented as mean±SD or no (%)

Table 2. Association between retinal vascular calibre and incident knee replacement for osteoarthritis

	Number at risk	Events (%)	HR (95% CI)	p	HR (95% CI)	p	HR (95% CI)	p
			Model 1*		**Model 2†**		**Model 3‡**	
Retinal arteriolar calibre								
Standardized retinal arteriolar calibre			1.23 (0.98-1.54)	0.07	1.25 (1.00-1.56)	0.05	1.27 (1.00-1.59)	0.05
Tertile 1: ≤165.3 µm	612	5.2	1.94 (0.98-3.83)	0.04	1.98 (1.00-3.92)	0.05	1.99 (1.00-3.96)	0.05
Tertile 2: >165.3-184.6 µm	614	5.4	2.02 (1.03-3.93)	0.06	2.02 (1.03-3.94)	0.04	1.97 (1.00-3.85)	0.05
Tertile 3: >184.6 µm	612	2.0	1.00		1.00		1.00	
Retinal venualr calibre								
Standardized retinal venular calibre			1.21 (0.95- 1.54)	0.12	1.22 (0.95-1.56)	0.11	0.86 (0.67-1.10)	0.22
Tertile 1: ≤195.0 µm	612	6.2	1.53 (0.86-2.70)	0.15	1.53 (0.87-2.72)	0.14	1.42 (0.80-2.53)	0.24
Tertile 2: >195.5-214.8 µm	614	3.3	0.79 (0.42-1.50)	0.47	0.77 (0.45-1.48)	0.45	0.77 (0.40-1.48)	0.43
Tertile 3: >214.8 µm	612	3.1	1.00		1.00		1.00	

*adjusted for sex and BMI; † adjusted for sex, BMI, physical activity, and HbA1c; ‡ adjusted for sex, BMI, physical activity, HbA1c, systolic blood pressure, total cholesterol and microalbuminuria

4.3 Summary findings from chapter 4

Two components of MetS, central obesity and hypertension and cumulative number of MetS components were associated with increased risk of severe knee OA independent of obesity. Neither cumulative MetS components nor individual component of MetS were associated with the risk of severe hip OA. Likewise, microcirculation measured by retinal vasculature has a role in knee OA. Particularly retinal arteriolar narrowing was associated with increased risk of knee replacement for OA.

Interventions targeted at controlling MetS and modifying microcirculation through lifestyle interventions and through pharmacologic treatment may play a role in the prevention and treatment of knee OA

Chapter 5: Serum 25-hydroxy-vitamin D concentrations and risk of hip osteoarthritis

Emerging evidence suggests that subtle changes in bone shape or geometry itself could lead to abnormal joint loading and predispose to OA (48, 49, 146, 147). Serum 25(OH)D plays an essential role in promoting calcium absorption to enable mineralization and promote healthy bones (45). As a result, serum 25(OH)D changes bone microstructure, bone mass and/or BMD (122). There is also evidence that vitamin D receptors are present in cartilage (148). As a result, vitamin D status might affect the development and progression of OA, especially hip OA, either directly via its effects on cartilage, or indirectly via its effects on bone related to bone shape change (54).

This chapter addresses issue related to the relationship between serum 25(OH)D concentration and its relationship with hip OA. It includes one manuscript examining the circulating serum 25(OH)D concentration and joint replacement for severe hip OA.

Declaration for Thesis Chapter 5

Declaration by candidate

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

Manuscript: Is serum level of 25-hydroxyvitamin D associated with the risk of hip replacement for osteoarthritis? Results from a prospective cohort study. (Under Review)

Nature of contribution	**Extent of contribution (%)**
Study design, data analysis and interpretation, manuscript development and preparation	75%

The following co-authors contributed to the work. Where co-authors are students at Monash University, the extent of their contribution in percentage terms is stated:

Name	**Nature of contribution**	**Extent of contribution (%) for student co-authors only**
Daly	Data collection and manuscript editing	
Wang	Data analysis and interpretation, manuscript development and preparation	
Shaw	Data collection and manuscript editing	
Magliano	Data collection and manuscript editing	
Graves	Data collection and manuscript editing	
Ebling	Data interpretation and manuscript editing	
Wluka	Data interpretation and manuscript editing	
Cicuttini	Study design, data analysis and interpretation, and manuscript editing	

The undersigned hereby certify that the above declaration correctly reflects the nature and extend o the candidate's and co-author' contributions to this work

Candidate's Signature		**Date**
Main Supervisor's Signature		**Date**

5.1 Serum concentration of 25-hydroxy-vitamin D and risk of hip osteoarthritis

Vitamin D plays an important role in skeletal mineralization and is associated with changing the bone microstructure; bone mass and/or BMD (122). Based on these effects it has been hypothesized that vitamin D status might affect the development and progression of OA, especially hip OA. Findings from a number of epidemiological studies with regard to the risk of hip OA in relation to serum 25(OH)D concentrations are inconclusive, with two studies showing beneficial effect of serum 25(OH)D concentrations on hip OA (54, 124) while others showing no effect (57, 58). The aforementioned studies had a number of limitations, including small sample size (54, 58, 124), cross-sectional study design (124), and failure to adjust for season of blood collection, which affects serum 25(OH)D concentrations significantly (54).

This prospective cohort study was carried out to explore whether serum 25(OH)D concentrations were associated with the risk of severe hip OA requiring hip joint replacement in a large cohort of Australian adults, taking into account relevant confounders.

Hussain SM, Daly RM, Wang Y, Shaw JE, Magliano DJ, Graves S, Ebeling PR, Wluka AE, Cicuttini FM. Is serum level of 25-hydroxyvitamin D associated with the risk of hip replacement for osteoarthritis? Results from a prospective cohort study. (Under Review)

In this study it was found that higher serum 25(OH)D concentrations was associated with an increased risk of hip OA requiring hip joint replacement in males but not in females. This study also adds to the ongoing debate regarding the optimal serum concentrations of vitamin D for skeletal health particularly in the setting of widespread vitamin D supplementation.

Is serum level of 25-hydroxyvitamin D associated with the risk of hip arthroplasty for osteoarthritis? Results from a prospective cohort study

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Key words: 25-hydroxy vitamin D, osteoarthritis, hip arthroplasty

ABSTRACT

Objective To determine whether serum 25-hydroxy-vitamin D [25(OH)D] concentrations were associated with the risk of hip arthroplasty for osteoarthritis (OA).

Design Prospective cohort study.

Setting Australian Diabetes, Obesity and Lifestyle (AusDiab) Study.

Population 9,135 participants who had serum 25(OH)D measured in 1999-2000 and were aged ⩾40 years at the commencement of arthroplasty data collection.

Main outcome measures The incidence of hip arthroplasty for OA during 2002 to 2011 was determined by linking cohort records to the Australian Orthopaedic Association National Joint Replacement Registry. Cox proportional hazard regression models were used to estimate the hazard ratio (HR) with 95% confidence intervals (CI) for the incidence of hip arthroplasty due to OA associated with serum 25(OH)D concentration, with age as the time scale. Interactions were tested for sex, obesity, physical activity and age. Since an interaction with sex was identified, all analyses were performed for males and females separately, and adjusted for body mass index, smoking status, ethnicity, physical activity, season of blood collection, latitude, area-level disadvantage, diabetic status and hypertension.

Results 201 hip arthroplasties for OA were identified (males n=90; females n=111). In males, a one SD increase in 25(OH)D concentration was associated with a 25% increased incidence of hip arthroplasty for OA (HR 1.25, 95% CI 1.02 to 1.56). A dose response relationship was observed for the incidence of hip arthroplasty for OA with elevating quartiles of 25(OH)D concentration in men (P for trend 0.04). No significant association was observed in women (HR 1.10 per SD increase in 25(OH)D concentration, 95% CI 0.87 to 1.39).

Conclusion Higher serum 25(OH)D concentrations were associated with an increased risk of hip arthroplasty for OA in males, but not in females. The mechanism for the association warrants further investigation.

What this paper adds

What is already known on this subject

- Vitamin D is thought to influence the course of hip OA via its known role in skeletal health.
- However evidence from epidemiological studies with regard to the association between serum 25(OH)D concentrations and the risk of hip OA is conflicting.

What this study adds

- Contrary to the hypothesis of beneficial effect of vitamin D, our findings that higher serum 25(OH)D concentrations predicted an increased incidence of hip arthroplasty for OA in males, but not in females adds to the ongoing debate about optimal serum concentrations of vitamin D for skeletal health particularly in the setting of widespread vitamin D supplementation.

Introduction

Osteoarthritis (OA) is the most common form of arthritis, causing pain and functional disability. Symptomatic hip OA represents a substantial burden with one in four people developing this condition in their lifetime.[1] Meanwhile, the number of years lived with disability for hip OA has increased over the last two decades due in part to the increases in life expectancy.[2] Bone is a dynamic tissue undergoing constant remodelling, with an increasing body of evidence highlighting the role of bone in the pathogenesis of OA. It is clear from a number of radiographic and biochemical studies that bone and cartilage pathology are linked in OA.[3 4] For example the geometry of the hip joint itself is an important risk factor for OA, with subtle architectural changes predating the radiographic appearance of hip OA in up to 90% of cases.[5 6]

Currently there is ongoing debate regarding optimal serum concentrations of 25-hydroxy-vitamin D [25(OH)D] for skeletal health.[7 8] Vitamin D plays an essential role in promoting calcium absorption to enable mineralization and promote healthy bones as well as improve lower extremity muscle function and reduce falls risk.[9] There is also evidence that vitamin D has multiple biological functions in cartilage via vitamin D receptors.[10] While articular cartilage loss is a key feature of OA, it is now recognised that OA is a disease of the whole joint, including bone. Thus, the interaction between bone and cartilage is believed to be central to cartilage homeostasis. As a result, it has been hypothesized that vitamin D status might affect the development and progression of OA, especially hip OA, either directly via its effects on cartilage, or indirectly via its effects on bone.[11] However, the findings from a number of epidemiological studies with regard to the risk of hip OA in relation to serum 25(OH)D concentrations are inconclusive. A summary of these studies is provided in Table 1. Briefly, while some studies have reported that low serum 25(OH)D concentrations are

associated with an increased prevalence and incidence of hip OA,[11 12] others have shown no effect.[13 14] Unfortunately, many of these studies had a number of limitations which make interpretation of the findings difficult, including small sample size,[11-13] cross-sectional study design,[12] and failure to adjust for season of blood collection, which affects serum 25(OH)D concentrations significantly.[11] Moreover, these studies have used different methods to define hip OA, such as radiographic hip OA,[11 12] clinical examination by physicians,[13] or the International Classification of Diseases codes.[14] One method for defining OA is to use arthroplasty, which identifies severe clinical hip OA relevant to the symptomatic disease burden and economic impact of OA.[15 16] Therefore, the aim of this prospective cohort study was to determine whether serum 25(OH)D concentrations were associated with the risk of hip arthroplasty for OA in a large cohort of Australian adults, taking into account relevant confounders.

Patients and Methods

Study participants

The Australian Diabetes, Obesity and Lifestyle (AusDiab) Study is a national, population-based cohort study of 11,247 people, aged ⩾25 years, recruited by a stratified cluster sampling method, involving seven strata (six states and the Northern Territory) and clusters based on census collector districts, during 1999 to 2000.[17] The study was approved by the International Diabetes Institute Ethics Committee and the Monash University Human Research Ethics Committee.[17] All participants gave written informed consent. For the current study, participants were restricted to those aged ⩾40 years at the commencement of arthroplasty data collection (January 2002) by the Australian Orthopaedic Association National Joint Replacement Registry (AOA NJRR), as arthroplasty for the treatment of OA is very uncommon under this age.[18] Of the 11,247 AusDiab participants that attended a

biomedical evaluation and provided a fasting blood sample in 1999/2000, a total of 2,112 were excluded as they were aged <40 years or had the first recorded arthroplasty as a revision surgery, leaving 9,135 participants eligible for the current study. The data linkage study was approved by the Alfred Hospital Ethics Committee, the University of Adelaide and Monash University Human Research Ethics Committees.

Demographic, lifestyle factors, and socioeconomic position assessments

Demographic and lifestyle data, including date of birth, gender, ethnicity (Europid vs non-Europid), smoking (current-, ex- or never), and leisure time physical activity (minutes per week), were collected in 1999 to 2000 by trained interviewers using standardised questionnaires as reported previously.[17] Physical activity was assessed using Active Australia Survey, which predominantly assesses leisure-time physical activity which includes duration and frequency of walking for recreation or transport; other moderate activity e.g. lawn bowls, golf, and gentle swimming; and vigorous activity e.g. gardening, tennis, jogging, cycling, and keep-fit exercise) during the previous week.[19 20] Total physical activity represents the sum of the time spent walking (if continuous and 10 minutes or more), the time spent doing other moderate intensity activities, plus the time spent participating in vigorous intensity activity. According to the current public health physical activity guidelines recommendation, participants were categorized as insufficiently active (reporting some moderate- or vigorous-intensity physical activity but less than 150 minutes in the last week), or sufficiently active (reporting 150 minutes or more activity at a moderate- or vigorous-intensity level in the last week.[19 20] This physical activity measure has been shown to provide acceptable precision [intraclass correlation = 0.59, 95% confidence interval (CI) 0.52 to 0.65] and validity (criterion validity = 0.3) estimates of exercise among adults.[20 21] Data on vitamin D supplement use was not obtained.

Area-level socioeconomic disadvantage was estimated using the Index of Relative Disadvantage code from the Socioeconomic Indexes for Areas (SEIFA). The index was developed by the Australian Bureau of Statistics. This is a summary measure from a group of 20 variables (related to education, income, employment, family composition, housing benefits, car ownership, ethnicity, English language proficiency, residential overcrowding) displaying dimensions of social disadvantage.[22] The index is constructed as such that high values reflect areas with high socioeconomic position (relative advantage) and low values reflect areas with low socioeconomic position (relative disadvantage).

Anthropometric and clinical measurements

Height was measured to the nearest 0.5 cm without shoes using a stadiometer. Weight was measured without shoes and in light clothing to the nearest 0.1 kg using a mechanical beam balance. Body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared.[17] Blood pressure was measured with Dinamap/mercury sphygmomanometer.[17] Hypertension was defined as blood pressure >140/90 mmHg or current use of antihypertensive medication.[17]

Fasting plasma glucose (FPG) and 2-hour plasma glucose (2-hour PG) were measured with an Olympus AU600 analyzer (Olympus Optical, Tokyo, Japan).[17] Participants were classified as having known diabetes mellitus if they reported having doctor diagnosed diabetes mellitus and were either taking hypoglycaemic medication or had a FPG $\geqslant$7.0 mmol/L or 2-hour PG $\geqslant$11.1 mmol/L. Participants not reporting having diabetes mellitus but who had a FPG $\geqslant$7.0 mmol/L or 2-hour PG $\geqslant$11.1 mmol/L were classified as having newly diagnosed diabetes mellitus.

Assessment of vitamin D status

Blood samples were stored at –70°C until assayed. As previously reported,[23] serum 25(OH)D was measured in the entire AusDiab population at baseline (1999/2000) using the Liaison 25(OH)D TOTAL (Liaison25OHD) (DiaSorin Inc., Stillwater, MN), a direct competitive chemiluminescent immunoassay with an interassay coefficient of variation of 7.0% at 45 nmol/L and 6.3% at 93 nmol/L. In 210 samples where fasting serum were not available, fluoride oxalate plasma (fasting plasma n = 190; 2-h plasma post-OGTT n = 20) was used.[24] There was excellent agreement between serum 25(OH)D concentrations collected from both tubes (n = 100): fluoride oxalate plasma 25(OH)D =0.97 x serum 25(OH)D + 2.5, r^2=0.89.[24] Season of blood sampling was divided into: Summer (Dec to Feb), Autumn (Mar to May), Winter (June to Aug) and Spring (Sept to Nov).[25] The latitude of each blood collection centre was determined using the Google GPS tool (range 12 to 43°S).[24]

Identification of incident primary hip arthroplasty

Cases were identified from the AOA NJRR as those who underwent a primary hip arthroplasty. Detailed information is available on prostheses, patient demographics, type and reason for arthroplasty. Data are collected from both public and private hospitals and validated using a sequential multi-level matching process against State and Territory Health Department unit record data.[26] Following the validation process and retrieval of unreported records, the Registry collects an almost complete set of data relating to hip arthroplasty in Australia.[26] Matching of AusDiab participants using first name, surname, date of birth, and gender, to the AOA NJRR in order to identify those who had a arthroplasty performed between 1 January 2002 and 31 December 2011 was performed using U.S. Bureau of the Census Record Linkage Software. This study examined the first hip arthroplasty with a contemporaneous diagnosis of OA, as recorded in the AOA NJRR.[27] If one person had

multiple arthroplasties, such as bilateral hip arthroplasties, or both knee and hip arthroplasties, the first recorded procedure was considered the event.

Statistical analysis

Descriptive information for each of the variables was derived and normality of the continuous data was verified before analysis. Independent samples t-tests for continuous variables or chi-squared tests for categorical variables were used to compare the characteristics of the participants with and without hip arthroplasty for OA. Cox proportional hazard regression models were used to estimate the hazard ratio (HR) with 95% CI for the incidence of hip arthroplasty due to OA associated with serum 25(OH)D, with age as the time scale. Follow-up for arthroplasty (calculation of person-time) began at 1 January 2002, and ended at the date of first hip arthroplasty for OA or date of censoring. Participants were censored at either the date of first hip arthroplasty for indications other than OA, the date of death, or end of follow-up (31 December 2011), whichever came first. To test whether associations of serum 25(OH)D with hip arthroplasty risk were modified by sex, obesity, physical activity (sufficient vs insufficient) or age (<65 years vs ≥65 years), interactions were fitted, and tested using the likelihood ratio test. Since an interaction with sex was identified, all analyses were performed for males and females separately, and adjusted for BMI, smoking status, ethnicity, physical activity, season of blood collection and latitude (model 1). To examine whether the association could be explained by the socioeconomic position and comorbidity; area-level disadvantage, diabetic status and hypertension were additionally adjusted for in model 2. As walking and gardening are outdoor activities which might affect the serum 25(OH)D concentration, additional modelling was done including walking and gardening time instead of physical activity. Serum 25(OH)D was standardized so that the HRs represent the effect of a one-standard-deviation (SD) difference in 25(OH)D. Serum 25(OH)D was also

categorized into gender specific quartiles. The lowest quartile was used as the referent category. Linear association between 25(OH)D and hip arthroplasty risk was examined using the likelihood ratio test. The P-value for trend was calculated by assigning the participants the median value of each serum 25(OHD) category and including this as a continuous variable in the model. To ensure that the result was not driven by extremely low 25(OH)D concentrations, additional analysis was done excluding those with vitamin D deficiency (25(OH)D <25 nmol/L, n=276). All statistical analyses were performed using Stata 12.0 (StataCorp LP., College Station, TX, USA).

Results

Over the 9.1 (SD 2.7) years of follow-up, 201 hip arthroplasties for OA were identified (men n=90; women n=111). The characteristics of the participants with and without hip OA are presented in Table 2. Both male and female participants who received a hip arthroplasty for OA were older, heavier, and more likely to be Europid and hypertensive.

Since there was evidence that sex modified the associations between serum 25(OH)D concentration and hip arthroplasty risk (p=0.05), men and women were examined separately (Table 3). In men, a one SD increase in 25(OH)D was associated with a 25% increased risk of hip arthroplasty for OA(HR 1.25, 95% CI 1.02 to1.56). When the 25(OH)D concentration was examined as a categorical variable using quartiles, a dose response relationship was observed such that the risk of hip arthroplasty for OA was increased across increasing quartiles of serum 25(OH)D concentration (P for trend =0.04), independent of age, BMI, ethnicity smoking status, physical activity, season of blood collection and latitude, hypertension and diabetes, and socioeconomic position. Additional modelling with walking and gardening time instead of physical activity did not change the association (HR 1.25, 95%

CI 1.01 to 1.54). In contrast, no associations were observed between serum 25(OH)D concentration and hip arthroplasty for OA in women (HR 1.10, 95% CI 0.87 to 1.39). When we excluded participants who had serum 25(OH)D concentrations <25 nmol/L, indicative of severe vitamin D deficiency, a one SD increase in 25(OH)D was associated with a 23% increased incidence of hip arthroplasty (HR 1.23, 95% CI 1.01 to 1.53) in men.

Discussion

We found that higher serum 25(OH)D concentrations predicted an increased risk of hip arthroplasty for OA in males but not in females, independent of age, BMI, smoking status, physical activity, season of blood collection and latitude, comorbidities and socioeconomic position. These results persisted even when those with low concentrations of 25(OH)D were excluded.

Strengths and limitations

The strengths of our study include its prospective design, large sample size, measurement of serum 25(OH)D concentrations in the entire population at baseline, adjustment of season and latitude, and the validation and completeness of AOA NJRR data,[26] However, there are a number of limitations. Serum 25(OH)D was only measured at baseline, which may not reflect long term vitamin D status. We defined OA based on arthroplasty. However, arthroplasty as the treatment of OA may be influenced by a number of factors such as access to health care, socioeconomic status, and patient preference,[28] in addition to disease severity. We performed the analysis on an age scale and adjusted for BMI, ethnicity, smoking status, physical activity, comorbidity and socioeconomic positioning to counter this issue. We have also compared the rate of hip arthroplasty in different socioeconomic position categories and found no difference (2.1% highest, 2.3% middle and 2.3% lowest tertile p=0.90). We did not

have arthroplasty data prior to 2002. This may have resulted in non-differential misclassification of hip arthroplasty, which is likely to bias the results to the null. Likewise, it is possible that there is residual confounding, for example vitamin D supplementation and activities that might influence hip injury on which data was not collected.

Strengths and weaknesses in relation to other studies, discussing important differences in results

At present, the data from previous observational studies is conflicting regarding the association between serum 25(OH)D concentrations and the risk of hip OA.[11-14] The findings from our study suggest that there is an adverse effect of higher concentrations of serum 25(OH)D on hip arthroplasty for OA in men, but not women. The Mini-Finland Health Examination Survey which was a 22 year follow-up study of 805 Finnish people,[13] and the Health 2000 Survey of Finland which was a 10 years follow-up study of 5,274 Finnish people,[14] both concluded that low concentrations of 25(OH)D do not contribute to the development of hip OA; though the Health 2000 Survey of Finland found a weak trend (p for trend 0.053) for higher concentration of serum 25(OH)D to be associated with increased risk of hip OA.[14] However, it is difficult to compare findings across studies because the baseline serum 25(OH)D concentrations in these two studies were approximately 12 to 25 nmol/L lower than that of our study (Table 1). Similarly, a recent systematic review found limited evidence to support that low concentrations of serum 25(OH)D were associated with incidence or progression of radiographic hip OA.[29] In contrast, low serum concentrations of 25(OH)D were found to be associated with increased incident radiographic hip OA characterized by joint space narrowing over an average period of 8 years in a random sample of 237 women from the Study of Osteoporotic Fractures.[11] Similarly, the Osteoporotic Fractures in Men Study (MrOs), which was a cross-sectional study from six areas of the US,

found that men with vitamin D deficiency were more likely to have prevalent radiographic hip OA.[12] However, both these studies were performed in people who were older (>70 years at baseline) than our population, and the Study of Osteoporotic Fractures did not adjust for season of blood collection, which is associated with the serum 25(OH)D concentration.

Meaning of the study: possible explanations and implications for clinicians and policymakers

The finding that there was an increased risk of hip arthroplasty for OA in men with higher serum 25(OH)D concentrations in this large prospective cohort study, after adjusting for potential confounding, is difficult to explain. We found that, men in the highest quartile of serum 25(OH)D concentration were of similar age and socioeconomic position (SEIFA score), more physically active, had lower BMI and were less likely to be diabetic and hypertensive than those in the lower quartiles. This suggests that the men in the highest quartile of serum 25(OH)D concentration were more likely to be healthy and fit for undergoing hip arthroplasty surgery for OA (Supplementary Table 1). Nevertheless, the rate of hip arthroplasty was similar across all the tertiles of the socioeconomic status (2.1% highest, 2.3% middle and 2.3% lowest tertile p=0.90) and in the diabetic and non-diabetic men (1.5% vs 2.2% p=0.24), it was higher in obese (non-obese vs obese, 1.9% vs 3.2% p=0.02) and in hypertensive men (non-hypertensive vs hypertensive, 1.7% vs 2.8% p=0.02).

The mechanism by which higher concentrations of serum 25(OH)D increase the incidence of hip arthroplasty for OA in men is unknown, but may be related to the positive association between concentrations of serum 25(OH)D and hip bone mass and/or BMD.[30] Hip bone shape, geometry, mass and BMD have all been shown to be important in the pathogenesis of hip OA.[5] Higher hip bone mass[31 32] and BMD[32 33], and greater bone size[34] have been

associated with an increased risk of hip OA through changing shape and size of the bone.[32] Moreover, those with hip OA have higher BMD at other skeletal sites, including femoral neck, lumbar spine and radius.[35] In addition, studies have shown that vitamin D has a biphasic effect on bone mass: either low or high concentrations can potentially accelerate bone resorption.[36 37] Indeed, it was found that 8.5% of the population with 25(OH)D concentrations >50nmol/L had osteomalacia,[38] a condition capable of producing subtle bone deformity[39 40] which might result in hip OA by affecting bone shape. Higher concentrations of serum 25(OH)D may increase the risk of OA via an effect on articular cartilage. The vitamin D receptor present in osteoblasts (the bone-forming cells), directly affect osteoblast growth and differentiation by stimulating bone formation and mineralization.[41] It was found that among the patients of hip OA, osteoblasts increased active bone formation.[42] Not only that, bioactive mediators produced by osteoblasts diffuse to the articular cartilage, resulting in cartilage degradation and thinning of the articular cartilage which ultimately can lead to OA.[43] Vitamin D activates matrix-degrading enzymes such as matrix metalloproteinase 13 (MMP 13) expression in articular cartilage.[44] MMP 13 targets type II collagen and aggrecan which are two major components of cartilage matrix for degradation, and facilitate cartilage erosion at the site of inflammatory arthritis.[44]

In our study population those who were in the higher quartile of the serum 25(OH)D concentration were more active. Thus, one possible explanation for the observed positive association between concentration of serum 25(OH)D and the risk of hip OA may be mediated through physical activity. Although time spent in leisure-time physical activity, or walking and gardening time, was included as a confounder, it is possible that physical activity might accelerate “wear and tear” of the hip joints, resulting in joint injury which ultimately will lead to hip OA. For example, occupational or leisure physical activity has been shown to

influence the incidence of self-reported physician-diagnosed OA of the knee and/or hip.[45] Another case-control study reported a positive association between prolonged regular sporting activity and the risk of hip arthroplasty for OA.[46] Our study population were drawn from the community, and thus it is less likely that they are involved in regular sports activity. However, we do not have data on activities that might influence which is an important risk factor for hip OA such as the occupational activity.

The reason for the finding that there was detrimental effect of higher serum 25(OH)D on the incidence of hip OA in males, but not in females, is not clear. However, we found that the concentrations of serum 25(OH)D were significantly different between males and females (68.6±24.8 nmol/L vs. 57.3±22.1 nmol/L, p=<0.001). It is possible that this gender difference in serum 25(OH)D may influence other biochemical or hormonal measures related to bone and/or cartilage health. For instance, sex steroids have been found to be associated with bone geometric structure change in the hip.[47] Besides, there are mutual interaction between vitamin D and estrogenic compounds,[48] which might also explain our finding.

Unanswered questions and future research

Owing to a number of limitations, previous observational studies have reported inconclusive findings regarding the association between serum 25(OH)D concentrations and the risk of hip OA.[11-14] The hypothesis that vitamin D has beneficial effect on skeletal health and the results from some observational studies showing vitamin D insufficiency to be associated with a wide variety of disorders such as fractures, cardiovascular disease, and cancer have resulted in the use of widespread vitamin D supplementation.[49] Recent findings from sequential meta-analysis suggest that vitamin D supplementation with or without calcium does not reduce adverse skeletal outcomes in community-dwelling individuals.[50] Our study adds to the

ongoing debate regarding the optimal serum concentrations of vitamin D for skeletal health particularly in the setting of widespread vitamin D supplementation.

In conclusion, this study showed that higher serum 25(OH)D concentrations predicted an increased risk of hip arthroplasty for OA in males but not in females. The mechanism underlying the association warrants further investigation.

A competing interest declaration

"All authors have completed the Unified Competing Interest form at www.icmje.org/coi_disclosure.pdf (available on request from the corresponding author) and declare that (1) [SMH, RMD, YW, JES, DJM, SG, PRE, AEW, FMC] have no support from any company for the submitted work; (2) [SMH, RMD, YW, JES, DJM, SG, PRE, AEW, FMC] have no relationships with any companies that might have an interest in the submitted work in the previous 3 years; (3) their spouses, partners, or children have no financial relationships that may be relevant to the submitted work; and (4) [SMH, RMD, YW, JES, DJM, SG, PRE, AEW, FMC] have no non-financial interests that may be relevant to the submitted work."

Contributors: YW and FMC were involved in conception and design of the study. SMH was involved in statistical analysis and interpretation of the data, and drafted the manuscript. YW was involved in cleaning and merging the datasets and interpretation of the data. RMD, AEW and PRE were involved in the interpretation of the data. JES, DJM, RMD and SG were involved in the acquisition of the data. All authors reviewed the manuscript and approved the final manuscript. All authors had full access to all of the data in the study. FMC is the guarantor.

Ethical approval: The AusDiab study was approved by the International Diabetes Institute ethics committee. The current data linkage study was approved by the Alfred Hospital Ethics Committee, and the University of Adelaide and Monash University Human Research Ethics Committees.

All participants gave written informed consent.

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Table 1. Association between serum 25(OH)D concentrations and hip osteoarthritis from 4 published studies

Author and Year	Study design and time of recruitment	Participants	Level of 25(OH)D	Measurement of outcome	Adjustment of confounders	Conclusion
Lane et al. 1999[11]	Cohort study Recruited from 1986 to 1988	Subjects (n=237) were participants in the Study of Osteoporotic Fractures, a study of women aged 71 years at baseline from Baltimore, Minneapolis, Portland and the Monongahela Valley of the US	Radiographic hip OA: 62.4±24 nmol/L No radiographic hip OA: 67.1±20.0 nmol/L	Joint space narrowing (scale 0-3), osteophytes (scale 0-3) and a summary grade of hip radiographic OA	Age, site, weight, calcaneal bone mineral density, vitamin D supplement, self-reported health status, weekly kilocalories expended exercising, and hours sedentary per day	Low serum 25(OH)D is associated with incident changes of radiographic hip OA characterized by joint space narrowing.
Chaganti et al. 2010[12]	Cross sectional study Recruited from 2000 to 2002	Subjects (n=1,104) were elderly men aged 77.2±5.3 years at baseline from the Osteoporotic Fractures in Men Study from Birmingham, Minneapolis, Palo Alto, Pittsburgh, Portland and San Diego of the US	Radiographic hip OA: 64.9±19.5 nmol/L No radiographic hip OA: 58.4±16.7 nmol/L	Joint space narrowing (scale 0-4), osteophytes (scale 0-3), cysts (scale 0-3), subchondral sclerosis (scale 0-3), and femoral head deformity (scale 0-3)	Age, clinic, season of blood collection, self-reported hip pain, timed 6-meter walk, presence of 1 coexisting medical condition, and self-rated health	Men with 25(OH)D deficiency were twice as likely to have prevalent radiographic hip OA.

Author and Year	Study design and time of recruitment	Participants	Level of 25(OH)D	Measurement of outcome	Adjustment of confounders	Conclusion
Konstari et al. 2012[13]	Cohort study Recruited from 1978 to 1980	Participants (n=805) from the population register representative of the Finnish adults aged ≥30 years: the Mini-Finland Health Examination Survey	Men: 46.8 nmol/L Women: 45.5 nmol/L	Diagnosed on the basis of a standardized clinical examination by physicians	Age, gender, education, BMI, physical workload, smoking, leisure time physical activity, injuries, and season of blood collection.	The results do not support the hypothesis that a low serum 25(OH)D level contributes to the development of hip OA.
Konstari et al. 2014[14]	Cohort study Recruited from 2000 to 2001	Nationally representative population (n=5,274) of adults drawn from the Health 2000 Survey aged ≥30 years	Men: 44.7±16.9 nmol/L Women: 45.0±16.6 nmol/L	Identified using International Classification of Diseases, 10th revision (ICD-10), codes M16 and M17.	Age, gender, BMI, physical workload, education, smoking, leisure time physical activity, time of serum collection, injuries and difficulty in walking due to discomfort or trouble in the knee or hip	The results do not support the hypothesis that low levels of serum 25(OH)D contribute to the development of hip OA.

25(OH)D = 25-hydroxy vitamin D, OA = osteoarthritis, BMI = Body mass index

Table 2. Baseline characteristics of participants with and without hip OA over the follow-up period

	Hip OA	No Hip OA	P value
Males			
No	90	4,052	
Age, years	60.2 (10.2)	56.0 (12.0)	<0.001
Europids, n (%)	88 (97.8)	3,647 (90.1)	0.02
Area-level disadvantage (in lowest tertile %)	34 (38.6)	1,276 (32.0)	0.33
BMI, kg/m2	28.6 (3.5)	27.3 (4.1)	0.003
Current/ex-smoker, n (%)	9 (11.0)	633 (16.0)	0.18
Physical activity, min/week	250 (263)	316 (304)	0.09
Walking, times/week	3.4 (0.4)	4.0 (0.1)	0.27
Gardening, times/week	1.1 (0.2)	0.8 (0.0)	0.27
Blood collection time, n (%)			0.004
Summer (Dec-Feb)	4 (4.4)	457 (11.3)	
Autumn (Mar-May)	35 (38.9)	954 (23.5)	
Winter (June-Aug)	26 (28.9)	1,354 (33.4)	
Spring (Sept-Nov)	25 (27.8)	1,287 (31.8)	
Latitude by region, n (%)			0.006
$<30^0$	12 (13.3)	1,058 (26.1)	
30^0-35^0	39 (43.3)	1,755 (43.3)	
$>35^0$	39 (43.3)	1,239 (30.6)	
Diabetes, n (%)	7 (7.8)	477 (11.9)	0.24
Hypertension, n (%)	49 (54.5)	1,703 (42.3)	0.02
Serum 25(OH)D, nmol/L	71.6 (21.9)	68.6 (24.8)	0.24

	Hip OA	No Hip OA	P value
Females			
No	111	4,853	
Age, years	62.2 (10.7)	55.6 (12.2)	<0.001
Europids, n (%)	107 (96.4)	4,360 (89.9)	0.02
University/Further education, n (%)	33 (29.7)	1,470 (30.3)	0.90
Area-level disadvantage (in lowest tertile %)	32 (29.1)	1,595 (33.4)	0.64
BMI, kg/m2	28.0 (5.2)	27.0 (5.5)	0.07
Current/ex-smoker, n (%)	16 (14.7)	596 (12.5)	0.50
Physical activity, min/week	218 (265)	223 (282)	0.83
Walking, times/week	2.9 (0.3)	3.4 (0.1)	0.24
Gardening, times/week	0.6 (0.1)	0.5 (0.0)	0.33
Blood collection time, n (%)			0.14
Summer (Dec-Feb)	14 (12.6)	560 (11.5)	
Autumn (Mar-May)	35 (31.5)	1,101 (22.7)	
Winter (June-Aug)	32 (28.8)	1,589 (32.7)	
Spring (Sept-Nov)	30 (27.0)	1,603 (33.0)	
Latitude by region, n (%)			0.29
$<30^0$	30 (27.0)	1,286 (26.5)	
30^0-35^0	42 (37.8)	2,156 (44.4)	
$>35^0$	39 (35.1)	1,411 (29.1)	
Diabetes, n (%)	9 (8.2)	403 (8.4)	0.93
Hypertension, n (%)	54 (48.6)	1,688 (35.0)	0.003
Serum 25(OH)D, nmol/L	57.2 (20.6)	57.3 (22.1)	0.96

Data are presented as mean ± SD, or as percentage.

Table 3. Relationship of serum 25(OH)D concentrations with incidence of hip arthroplasty for osteoarthritis

	Model 1 Hazard ratio (95% CI)	P	Model 2 Hazard ratio (95% CI)	P
		Males		
Serum 25(OH)D (per SD)	1.25 (1.02, 1.56)	0.02	1.26 (1.01, 1.56)	0.04
Serum 25(OH)D				
1 (0 – 51 nmol/L)	1.00		1.00	
2 (52 – 65 nmol/L)	2.03 (1.01, 4.03)	0.04	2.02 (1.01, 4.06)	0.05
3 (66 – 81 nmol/L)	2.18 (1.07, 4.05)	0.03	2.17 (1.08, 4.06)	0.03
4 (⩾82 nmol/L)	2.30 (1.09, 4.82)	0.03	2.30 (1.09, 4.82)	0.03
P for trend	0.02		0.04	
		Females		
Serum 25(OH)D (per SD)	1.10 (0.87, 1.39)	0.44	1.11 (0.88, 1.41)	0.39
Serum 25(OH)D				
1 (0 – 41 nmol/L)	1.00		1.00	
2 (42 – 54 nmol/L)	1.30 (0.76, 2.23)	0.35	1.21 (0.70, 2.09)	0.50
3 (55 – 69 mmol/L)	1.10 (0.62, 1.95)	0.75	1.08 (0.61, 1.91)	0.80
4 (⩾70 nmol/L)	1.29 (0.71, 2.33)	0.40	1.29 (0.71, 2.34)	0.40
P for trend	0.54		0.49	

Model 1 adjusted for BMI, ethnicity, smoking status, physical activity, season of blood collection and latitude

Model 2 adjusted for model 1 and hypertension, diabetes, and Area-level disadvantage

5.2 Summary findings from chapter 5

It was found that higher serum 25(OH)D concentrations was associated with an increased risk of hip OA requiring hip joint replacement in males but not in females. This might be via change in hip bone shape, geometry, mass and BMD that are important in the pathogenesis of hip OA (35).

Chapter 6: Birth weight and risk of knee and hip osteoarthritis

Evidence is emerging that several adulthood diseases have their deep rooted link to the in utero programming. For example hypertension, insulin resistance, cardiovascular disease (63), and more recently reduced bone mass (64) has been linked to LBW and preterm birth. Preterm birth has been linked to a postural deformation of the legs with wide hip abduction and external rotation (126) and unstable hip type IIc (risky) and IId (decentralized) (127). Though bone mass and bone mineral contents, postural deformity has been linked to birth weight and preterm birth, no previous work has been done to examine the role of birth weight and preterm birth and the risk of knee and hip OA.

This chapter addresses issue related to the relationship between LBW and preterm birth and the risk of knee and hip OA. It includes one manuscript examining association between LBW and preterm birth and joint replacement for severe knee and hip OA.

Declaration for Thesis Chapter 6

Declaration by candidate

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

Manuscript: Association of low birth weight and preterm birth with the incidence of knee and hip arthroplasty for osteoarthritis. Arthritis Care & Research. (In press)

Nature of contribution	**Extent of contribution (%)**
Study design, data analysis and interpretation, manuscript development and preparation	75%

The following co-authors contributed to the work. Where co-authors are students at Monash University, the extent of their contribution in percentage terms is stated:

Name	**Nature of contribution**	**Extent of contribution (%) for student co-authors only**
Wang	Study design, data analysis and interpretation, and manuscript editing	
Wluka	Data interpretation and manuscript editing	
Shaw	Study design, data collection, and manuscript editing	
Magliano	Data collection and manuscript editing	
Graves	Data acquisition and manuscript editing	
Cicuttini	Study design, data analysis and interpretation, and manuscript editing	

The undersigned hereby certify that the above declaration correctly reflects the nature and extend o the candidate's and co-author' contributions to this work

Candidate's Signature		**Date**
Main Supervisor's Signature		**Date**

6.1 Low birth weight and preterm birth and risk of knee and hip osteoarthritis

Several studies have linked LBW and preterm birth with adverse metabolic outcomes in adulthood including hypertension, insulin resistance, cardiovascular disease (63, 149, 150), and more recently reduced bone mass (64). Recent research has revealed a metabolic link of knee OA. Conversely, mild hip dysplasia or DDH, may influence the development of hip OA in adulthood (35, 36). Data from an observational study demonstrated that formation of the acetabulum was incomplete among preterm babies (151). However, no previous study has explored the relationship between LBW and preterm birth with the risk of knee and hip OA.

This longitudinal study examined whether LBW and preterm birth were associated with the incidence of severe knee and hip OA requiring joint replacement.

Hussain SM, Wang Y, Wluka AE, Shaw JE, Magliano DJ, Graves S, Cicuttini FM. Association of low birth weight and preterm birth with the incidence of knee and hip arthroplasty for osteoarthritis. Arthritis Care & Research. 2014 Nov 3. doi: 10.1002/acr.22475. [Epub ahead of print]

This study found that LBW and preterm birth are associated with an increased risk of severe hip but not severe knee OA. This may be via the mechanisms of acetabular dysplasia and reduced bone mass. This finding highlight the importance that individuals born with LBW or preterm are at increased risk of hip OA in adult life so should be targeted as an “at risk group” for hip OA to be closely monitored.

Arthritis Care & Research
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Association of low birth weight and preterm birth with the incidence of knee and hip arthroplasty for osteoarthritis

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Short title: Low birth weight, preterm birth; and osteoarthritis

Word count: 2,452

Abstract

Objectives: Low birth weight (LBW) and preterm birth have been associated with adverse adult outcomes including hypertension, insulin resistance, cardiovascular disease and reduced bone mass. It is unknown whether LBW and preterm birth affect the risk of osteoarthritis (OA). This study aims to examine whether LBW and preterm birth were associated with the incidence of knee and hip arthroplasty for OA.

Methods: 3,604 participants of the Australian Diabetes, Obesity and Lifestyle Study who reported their birth weight and history of preterm birth and were aged more than 40 years at the commencement of arthroplasty data collection. The incidence of knee and hip replacement for osteoarthritis during 2002-2011 was determined by linking cohort records to the Australian Orthopaedic Association National Joint Replacement Registry.

Results: One hundred and sixteen participants underwent knee arthroplasty and 75 underwent hip arthroplasty for OA. Low birth weight (yes vs. no, HR 2.04, 95% CI 1.11-3.75, p=0.02) and preterm birth (yes vs. no, HR 2.50, 95% CI 1.29-4.87, p=0.007) were associated with increased incidence of hip arthroplasty independent of age, sex, BMI, education level, hypertension, diabetes, smoking and physical activity. No significant association was observed for knee arthroplasty.

Conclusions: Although these findings will need to be confirmed, they suggest that individuals born with LBW or preterm are at increased risk of hip arthroplasty for OA in adult life. The underlying mechanisms warrant further investigation.

Significance and Innovations:

- Low birth weight and preterm birth have been associated with adverse outcomes in adulthood including hypertension, insulin resistance, and cardiovascular disease and more recently reduced bone mass.
- It is unknown whether low birth weight and preterm birth affect the risk of osteoarthritis.
- This study finds out that low birth weight and preterm birth are risk factors for hip but not knee OA requiring arthroplasty.
- Individuals with low birth weight or preterm birth should be identified as an "at risk group" and targeted for close monitoring of hip OA.

Osteoarthritis (OA) is a major public health problem and the most common cause of disability, with OA of the knees and hips resulting in a total of 71.1 million years lived with disability in 2010, an increase of 64% since 1990 globally(1). Currently there are no registered disease-modifying OA drugs. Therefore understanding the risk factors for OA is important for improving prevention.

Low birth weight (LBW) and preterm birth have been associated with adverse outcomes in adulthood including hypertension, insulin resistance, cardiovascular disease(2), and more recently reduced bone mass(3). As an underlying mechanism, fetal nutrition in utero leading to reprogramming of the insulin-like growth factor 1 (IGF-1) axis has been proposed(4, 5). IGF-1 stimulates osteoblastic differentiation of mesenchymal stem cells and new bone formation, and thus maintains proper bone microarchitecture and mass(6).

Whether LBW and preterm birth affect the risk of OA is unknown. However, acetabular dysplasia has been linked with preterm birth(7, 8), and mild acetabular dysplasia is associated with an increased incidence of hip OA(9-11). There is increasing evidence suggesting that hip and knee OA are susceptible to different risk factors(12). Given the bony changes associated with LBW and preterm birth, we hypothesised that they would be associated with hip rather than knee OA.

Studies exploring knee or hip OA have generally defined OA using imaging modalities (12, 13). Another method for defining OA is based on arthroplasty(4, 16) which has been shown to be useful for identifying the potential risk factors for knee and hip OA(13). This definition signifies severe knee and hip OA which is relevant to the symptomatic disease burden and health economics. Thus the aim of this study was to determine whether LBW and preterm

birth were associated with the incidence of knee and hip arthroplasty as measures of severe OA in a prospective cohort study.

Methods

Study participants

The Australian Diabetes, Obesity and Lifestyle (AusDiab) Study is a national, population-based cohort study of 11,247 people, aged ≥25 years, recruited by a stratified cluster sampling method, involving seven strata (six states and the Northern Territory) and clusters based on census collector districts, during 1999-2000. In 2004-2005, a 5-year follow-up survey was conducted. All eligible participants included in the baseline survey were invited (n=10,788), of whom 7,157 (66.3%) responded (Figure 1). Detailed methods and response rates were described previously(13). The study was approved by the International Diabetes Institute ethics committee(13).

For the current study, participants were restricted to those aged ≥40 years at the commencement of data collection by the Australian Orthopaedic Association National Joint Replacement Registry (AOA NJRR), 1 January 2002, since arthroplasty as the treatment of OA is very uncommon under this age(14). Of the 7,157 participants, 950 were excluded as they were aged <40 years or had the first recorded arthroplasty as a revision surgery, leaving 6,207 participants eligible for the current study (Figure 1). The data linkage study was approved by the Alfred Hospital Ethics Committee, and the University of Adelaide and Monash University Human Research Ethics Committees.

Demographic, lifestyle factors, anthropometric and clinical measurement

Demographic and lifestyle data, including date of birth, gender, smoking, and physical activity were collected in 1999-2000 by trained interviewers using standardised questionnaires(13). Height was measured to the nearest 0.5 cm without shoes using a stadiometer. Weight was measured without shoes and in light clothing to the nearest 0.1 kg using a mechanical beam balance. Body mass index (BMI) was calculated in kilograms per square metre(13). Blood pressure was measured with Dinamap/mercury sphygmomanometer(13). Hypertension was defined as blood pressure >140/90 mmHg or current use of antihypertensive medication(13).

Blood was drawn after an overnight fast (≥9 h) for measurement of glucose followed by a 2-h 75 g oral glucose tolerance test. All specimens were analysed at a central laboratory. Fasting plasma glucose (FPG) and 2-h postload glucose were analysed by an automated glucose oxidase method (Olympus Optical Co. Ltd., Tokyo, Japan). Diabetes was defined if participants were on anti-diabetic medication, or they had FPG ≥7.0 mmol/L, or 2-h postload glucose ≥11.1 mmol/L(15).

Low birth weight and prematurity

At the 2004–2005 follow-up, participants were asked to state their birth weight and whether they were born two weeks or more preterm(2). Participants were also asked to indicate the likely accuracy and source of their answers(2). Of the 6,207 eligible participants, 3,604 reported a value for birth weight and preterm birth, with the others unable to give a value. More than 90% of respondents who reported a birth weight considered it to be “accurate” and only 6% were based on a “guess”; 80% obtained their birth weight from a family member (67% of participants had a living natural mother, and 46% a living natural father) and 10% from

medical records(2). Detailed information on accuracy and validity of self-reported LBW and preterm birth were previously described(2). LBW was defined as birth weight <2.5 kg. Participants who reported their birth weight were younger (49.6 ± 12.6 vs. 53.3 ± 15.8 years, $p < 0.001$), less likely to have diabetes (6.4% vs. 10.6%, $p < 0.001$) and hypertension (28.2% vs. 37.0%, $p < 0.001$), and had lower BMI (26.9 ± 4.9 vs. 27.1 ± 5.1 kg/m^2, p=0.05) compared with individuals who did not respond to the questionnaire or could not recall their birth weight. However, when the risk of arthroplasty of those who reported their birth weight versus those who did not was compared, there was no difference in risk of knee (2.8% vs. 2.8%) or hip (1.9% vs. 1.8%) arthroplasties for OA (all $p >0.80$).

Identification of incident primary knee and hip arthroplasty

Cases were identified from the AOA NJRR as those who underwent either a primary hip or a primary knee arthroplasty. Detailed information is available in the Registry on prostheses, patient demographics, type and reason for arthroplasty (such as OA, rheumatoid arthritis, fracture, etc.). Data are collected from both public and private hospitals and validated using a sequential multi-level matching process against State and Territory Health Department unit record data(16). Following the validation process and retrieval of unreported records, the Registry collects an almost complete set of data relating to hip and knee arthroplasty in Australia(16).

Matching of AusDiab participants using first name, surname, date of birth, and gender, to the AOA NJRR in order to identify those who had had a primary arthroplasty performed between 1 January 2002 and 31 December 2011 was performed using U.S. Bureau of the Census Record Linkage Software.

Definition of knee and hip OA

Knee or hip OA was defined as the first primary knee or hip arthroplasty with a contemporaneous diagnosis of OA, as recorded in the AOA NJRR(17). If one person had multiple arthroplasties, such as bilateral knee arthroplasty, bilateral hip arthroplasty, or both knee and hip arthroplasties, the first recorded procedure was considered the event.

Statistical analysis

Cox proportional hazard regression models were used to estimate the hazard ratios (HR) for knee or hip arthroplasty due to OA associated with LBW and preterm birth. Follow-up for arthroplasty (i.e. calculation of person-time) began in January 1, 2002, and ended at the date of first arthroplasty for OA or date of censoring. Participants were censored at either the date of first arthroplasty performed for indications other than OA, the date of death, or end of follow-up (i.e. December 31, 2011, the date that ascertainment of arthroplasty by NJRR was complete), whichever came first. LBW and preterm birth were analysed and modelled separately. Each analysis was adjusted for age, sex, and BMI, in model 1, as these are established risk factors for arthroplasty for OA(17). In model 2, the analyses were further adjusted for hypertension, diabetes, smoking status and physical activity. To test whether associations of LBW and preterm birth with arthroplasty risk were modified by obesity (BMI $\geq$ 30 kg/m^2) and sex, interactions were fitted, and tested using the likelihood ratio test. Tests based on Schoenfeld residuals and graphical methods using Kaplan-Meier curves showed no evidence that proportional hazard assumptions were violated for any analysis.

With the sample size of 3,604 participants who had complete data available on birth weight, our study had 80% power (at the 5% significance level, 2 sided significance) to detect a risk

ratio of 1.96 where the risk of knee or hip replacement in those without low birth weight or preterm birth was assumed to be 1.9% and the prevalence of low birth weight to be 9.0%.

All statistical analyses were performed using Stata 12.0 (StataCorp LP., College Station, TX, USA).

Results

One hundred and ninety one arthroplasties (116 knee arthroplasties and 75 hip arthroplasties) performed for OA were identified between January 1, 2002 and December 31, 2011. The mean follow up duration was 9.3 (SD 2.1) years. Descriptive characteristics of the study participants are presented in Table 1. Of the 3,604 participants, 122 participants had only low birth weight, 144 participants were only preterm and 135 participants had both low birth weight and preterm birth. The correlation between low birth weight and preterm birth was 0.45 (Pearson's correlation, p = <0.001). Participants who underwent a hip arthroplasty were more likely to be born with LBW or preterm than those who did not have a hip arthroplasty.

In age, sex and BMI adjusted analysis (model 1), both LBW [Hazard ratio (HR) 1.87, 95% confidence interval (CI) 1.02-3.41, p=0.04] and preterm birth (HR 2.41, 95% CI 1.25-4.66, p=0.009) were associated with increased incidence of hip arthroplasty for OA. The results remained significant after adding hypertension, diabetes, smoking and physical activity to the previous model, for both LBW (HR 2.02, 95% CI 1.10-3.73, p=0.02) and preterm birth (HR 2.53, 95% CI 1.30-4.92, p=0.006) (model 2). In contrast, neither LBW nor preterm birth was significantly associated with the incidence of knee arthroplasty for OA in unadjusted or adjusted analyses (Table 2).

There was no evidence that obesity or sex modified the associations between LBW or preterm birth and arthroplasty risk (all $p > 0.10$).

Discussion

This is the first study to report the relationship of LBW and preterm birth with the incidence of severe knee and hip OA requiring arthroplasty in a general population. LBW and preterm birth were associated with increased incidence of hip OA but not knee OA.

No previous studies have examined the association between LBW or preterm birth and the risk of OA. We found an association for hip OA requiring arthroplasty. The etiology of hip OA is multifactorial(18). Both congenital and developmental diseases of the hip, such as mild hip dysplasia, may influence the development of hip OA in adulthood(19, 20). The formation of the acetabulum is incomplete at birth in preterm babies(21). Preterm infants often develop a postural deformation of the legs which persists till early childhood(7), perhaps because of an underdeveloped or shallow, upwardly sloping acetabulum(22), decreased joint surface area(9), or because the ligaments holding the ball in place are too loose(7). These factors may influence the development of the hip, resulting in abnormal hip joint shape. The important role of hip bone shape and geometry in the aetiology of hip OA has been established(20). Premature and LBW babies represent a unique vulnerable population, in which bone growth and mineral acquisition are critical in regards to bone turnover(23). A case-control study similarly found reduced peak bone mass at the femoral neck in very low birth weight babies(3). There is emerging evidence that preterm birth and very low birth weight results in decrease in bone formation and increase in bone resorption(23, 24) that reduced osteoclast

apoptosis(25) and cartilage degeneration (26) which may be another potential pathways of development of hip OA.

Although we found a relationship of LBW and preterm birth with hip OA requiring arthroplasty, no relationship was observed for knee OA requiring arthroplasty. These differences support the notion of different susceptibility of these joints to various risk factors(12, 27). Thus, whilst bone shape and geometry are important in the aetiology of hip OA, these factors are less critical than soft tissue and other factors in the pathogenesis of knee OA(20). As LBW and preterm birth have significant impacts on bone and hip structure, this is biologically plausible(7).

Clarifying the mechanisms for the relationship between LBW and preterm birth and hip OA is important. LBW and preterm birth may result in abnormal hip development because these babies are born early and the acetabulum is underdeveloped(21, 22). Post-delivery, the hips are extended rather than being maintained in a flexed and abducted in utero position(28, 29). This altered hip position may potentially be responsible for an increased incidence or severity of acetabular dysplasia. If this is proven to contribute to the development of hip OA, then modifying hip position through postural support(28, 29) and perhaps the use of double nappies(30) may be beneficial for babies born with LBW or preterm, and they may need to be targeted for screening and early treatment of hip dysplasia. As the number of LBW and preterm births is increasing, if they are proven to be at increased risk of hip OA, the impact of proactive strategies to reduce hip OA, such as the prevention of obesity(31) will be greater.

The strengths of our study include its large sample size and prospective design. Although defining OA based on arthroplasty only identifies the tip of the iceberg of the true problem, it

signifies the severity of OA which is relevant to the symptomatic disease burden and health economics(31). Furthermore, the AOA NJRR data is validated and nearly complete regarding arthroplasty in Australia(16). The findings of our study need to be considered within the context of its limitations. Birth weights and preterm birth were self-reported. This might have resulted in recall and rumination bias. However, in this study, there is low scope of recall bias or rumination bias in birth weight or preterm birth in relation to arthroplasty. Birth weight and preterm birth data were collected during the first round of follow-up of the cohort in 2004-2005. The linkage component of the study in terms of joint replacement for OA was introduced in 2013. When people were asked about their birth status, there was no specific hypothesis that this would be associated with health outcomes including OA. We didn't ask people if they had low birth weight. We simply asked them about their weight at birth – many people would not know what a normal birth weight is, so sick people would be unlikely to be able to assign themselves an abnormal result. Previous studies reporting associations of birth weight with adult health have used this technique(2, 32-34). Self-reported birth weight in our study was 3.37±0.7 (mean±SD) kg, which was similar whether birth weight was obtained from family members or from medical records (3.35±0.6 vs. 3.37±0.7 kg; after adjustment for age and sex, P=0.36)(2). The birth weight of our study population is similar to the recent average Australian birth weight of 3.46 kg for boys and 3.33 kg for girls(35). Individuals with the highest and lowest birth weights tend to report normal birth weight(36) which will lead to underestimation of arthroplasty risk associated with LBW. Nevertheless, we found a significant association of LBW and prematurity with hip but not knee arthroplasty. It is unlikely that any misclassification would affect the relationship with arthroplasty at the hip but not the knee. For example, whilst participants who reported birth weight and prematurity had better health compared with those who did not respond to the questionnaire or could not recall their birth weight, the risks of both hip and knee arthroplasty were very similar. We did

not have arthroplasty data prior to 2002. It is possible that arthroplasties occurring before 2002 represent more rapidly progressive disease, and inclusion of those data in analysis may influence our findings. Only 1.5% of those who attended the baseline AusDiab study thought that they may have undiagnosed diabetes. Although this was higher than in those who did not participate, published data show that the absolute number was too small to have any measurable effect even on diabetes prevalence(37). Again, whether patients undergo arthroplasty as the treatment of OA may be influenced by a number of factors such as access to health care, physician bias, and patient-level factors(38), in addition to disease severity. Australia has a publicly-funded universal health system (Medicare) and people without private health insurance have access to joint replacement under this system. We have performed the analysis adjusted for age, sex, BMI, hypertension, diabetes, smoking and physical activity to counter this issue. Further adjustment for education and ethnicity did not change the results (data not shown). Moreover, it is possible that there is residual confounding. However, if residual confounding is the main explanation for the association between LBW and preterm birth and OA risk, we would expect the same association for knee and hip arthroplasty which was not the case as we observed differential effect of LBW and preterm on hip and knee OA.

LBW and preterm birth are associated with an increased risk of hip but not knee OA requiring arthroplasty. This may be via the mechanisms of acetabular dysplasia and reduced bone mass. Although these findings will need to be confirmed in other studies and the underlying mechanisms warrant further investigation, these data suggest that individuals born with LBW or preterm are at increased risk of hip arthroplasty for OA in adult life. Identifying individuals born with LBW or preterm as an “at risk group” for hip OA and targeting them for close monitoring and early interventions, may reduce the incidence of hip OA in later life.

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Table 1: Characteristics of study population

	No arthroplasty (n = 3,413)	Knee arthroplasty (n = 116)	Hip arthroplasty (n = 75)
Age at baseline (years)	51.8 (10.0)	59.7 (9.5)	59.0 (9.5)
Age at 2002 (years)	54.1 (10.0)	62.1 (9.5)	61.3 (9.3)
Female, n (%)	2,058 (60.3)	69 (59.5)	45 (60.0)
Body mass index (kg/m^2)	26.9 (4.9)	30.2 (5.3)	28.7 (4.4)
Hypertension, n (%)	981 (28.9)	68 (59.1)	36 (48.0)
Diabetes, n (%)	232 (6.7)	14 (12.3)	5 (6.7)
Smoking status, n (%)			
Non-smoker	1,929 (57.5)	68 (59.1)	39 (54.2)
Former smoker	1,031 (30.7)	36 (31.3)	25 (34.7)
Current smoker	395 (11.8)	11 (9.6)	8 (11.1)
Physical activity, n (%)			
Sedentary	484 (14.3)	26 (22.8)	15 (20.3)
Insufficient	1,027 (30.3)	40 (35.1)	19 (25.7)
Sufficient	1,878 (55.4)	48 (42.1)	40 (54.1)
Birth weight (kg)	3.4 (0.7)	3.5 (0.8)	3.3 (0.7)
Low birth weight, n (%)	303 (8.9)	11 (9.5)	13 (17.3)
Preterm birth, n (%)	270 (9.5)	8 (8.5)	11 (19.0)

Data presented as mean (SD) or no (%)

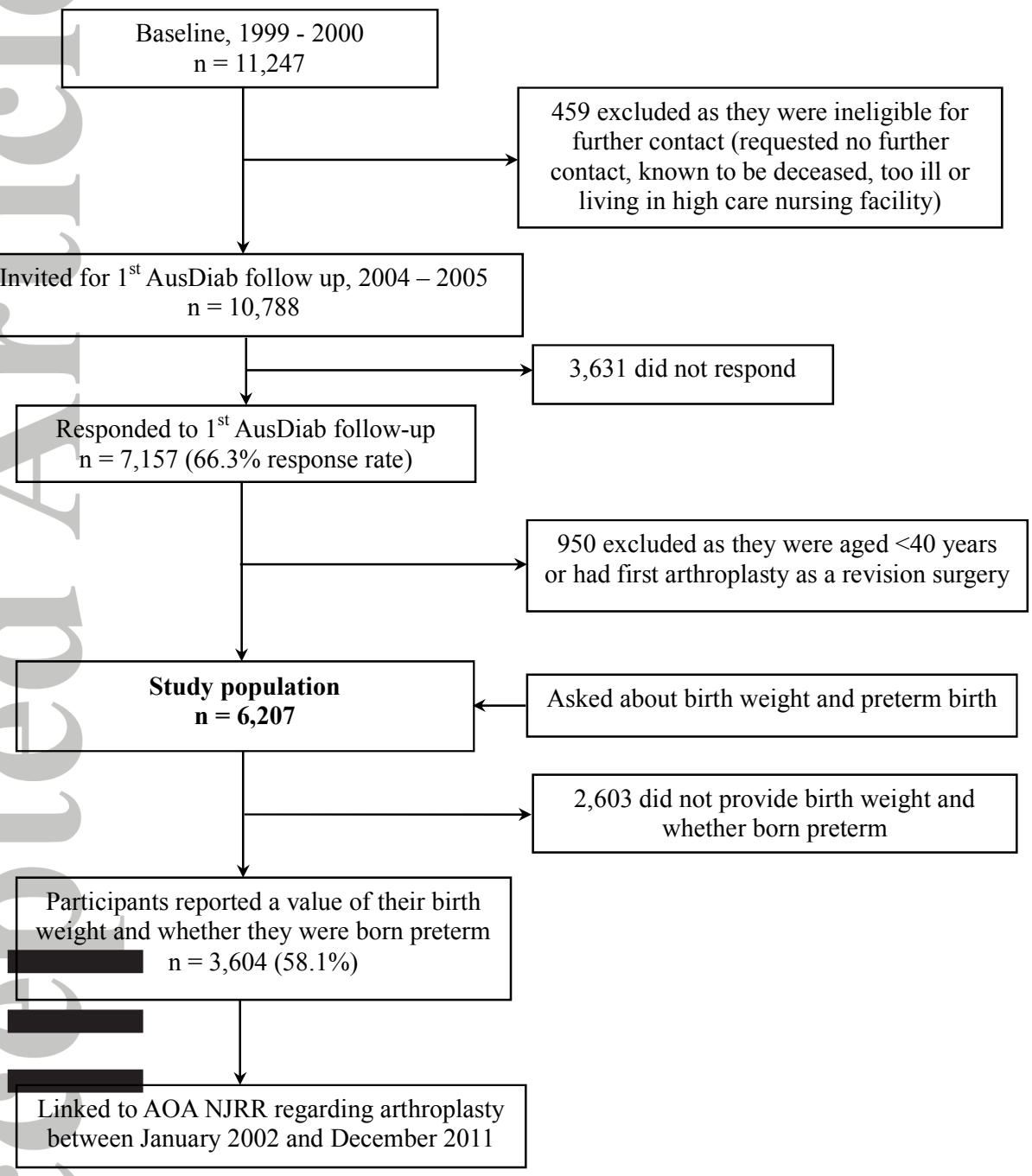
Table 2: Relationship of low birth weight and preterm birth with incidence of knee and hip arthroplasty for osteoarthritis

	Unadjusted Hazard ratio (95% CI)	P value	Adjusted Model 1* Hazard ratio (95% CI)	P value	Adjusted Model 2† Hazard ratio (95% CI)	P value
Knee arthroplasty						
Low birth weight	1.07 (0.58, 1.99)	0.83	0.93 (0.50, 1.73)	0.82	0.86 (0.45, 1.66)	0.65
Preterm birth	0.88 (0.43, 1.81)	0.73	0.97 (0.47, 2.00)	0.93	0.79 (0.36, 1.73)	0.56
Hip arthroplasty						
Low birth weight	2.14 (1.18, 3.90)	0.01	1.87 (1.02,3.41)	0.04	2.02 (1.10, 3.73)	0.02
Preterm birth	2.21 (1.15, 4.27)	0.02	2.41 (1.25, 4.66)	0.009	2.53 (1.30, 4.92)	0.006

*adjusted for age, sex and BMI

†adjusted for age, sex, BMI, hypertension, diabetes, smoking and physical activity

Figure 1: Flowchart of recruited participants from AusDiab study



6.2 Summary findings from chapter 6

This is the first study to report the relationship of LBW and preterm birth with the incidence of severe knee and hip OA requiring joint replacement surgery in a general population. LBW and preterm birth were associated with increased incidence of hip OA but not knee OA.

Chapter 7: Discussion

7.1 Main findings

This thesis is comprised of four major themes, focusing on the identification of novel and systemic risk factors for knee and hip OA.

The first theme examined the association between hormonal factors, including circulating sex hormones and 2D:4D ratio (an indicator of *in utero* exposure to sex hormones), and the risk of knee and hip replacement for OA. It was shown that, lower E2 concentration was a risk factor for knee OA and lower ASD concentration and greater SHBG concentration were risk factors for hip OA in women. There was an association between 2D:4D ratio and knee OA such that, lower 2D:4D was associated with an increased incidence of knee joint replacement for severe knee OA. No association of 2D:4D was observed with hip replacement for OA.

The second theme of the thesis explored the association between metabolic and vascular factors and the risk of knee and hip replacement for OA. It was found that the MetS and all its components were associated with increased incidence of severe knee OA requiring total knee replacement. Following adjustment for BMI, a measure of overall body size, the associations of central obesity, hypertension, and the MetS remained significant. With the accumulation of MetS components, the incidence of severe knee OA increased independent of BMI. In contrast, the incidence of severe hip OA was not related to either the individual or cumulative MetS components. For the vascular disease and risk of severe OA, this thesis found that narrower retinal arteriolar caliber, a unique way to assess the microcirculation predicted an increased risk of knee replacement for OA independent of age, sex, BMI, physical activity, HbA1c and other cardiovascular risk factors.

The third theme of this thesis examined whether circulating concentration of 25(OH)D was associated with the risk of hip replacement for severe OA. It was found that, serum concentration of 25(OH)D related to bone mass and/or BMD, predicted an increased risk of hip replacement for OA in males but not in females, independent of age, BMI, smoking status, physical activity, season of blood collection and latitude, comorbidities and socioeconomic position.

The fourth theme of this thesis examined whether LBW and premature birth were associated with the risk of knee and hip replacement for severe OA. It was found that, LBW and preterm birth probably via the mechanisms of acetabular dysplasia and reduced bone mass were linked to severe hip OA requiring hip joint replacement. However, LBW and preterm birth were not associated with the risk of severe knee OA.

Taken together, these results suggest the consistent finding that, risk factors for knee and hip OA differ. It was shown that, lower circulating concentration of E2 and lower 2D:4D and individual as well as cumulative components of MetS, microcirculation assessed by retinal arteriolar caliber were associated with increased risk of severe knee OA. Likewise, lower circulating concentration of ASD and higher circulating concentration of SHBG, LBW and preterm birth, and higher circulating concentration of 25(OH)D were associated with increased risk of hip OA.

7.2 Hormonal factors and risk of knee and hip osteoarthritis

The high incidence of OA in women after 50 years of age suggests that, sex hormones might have an involvement in the pathogenesis of the disease. Due to inconsistency of the findings from previous studies and paucity of studies investigating all the endogenous sex hormones in the same population, it is hard to determine the role of sex hormones in relation to knee and hip OA. 2D:4D a marker of *in utero* sex hormone exposure has shown inconsistent associations with knee and hip OA and there is no previous longitudinal study to report this association.

Though case control studies showed inconsistent findings for the relationship between hormone replacement therapy (HRT) and risk of OA (69, 71, 75, 77), two cohort studies showed beneficial effect of HRT in relation to reduced incidence and progression of knee OA (72) and decreasing prevalence of hip OA (76). It has been speculated that, this observed beneficial finding might be confounded by the healthy habits of HRT users, which might protect them from disease (3). The number of studies examining the relationship between endogenous sex steroids and the pathogenesis of OA are limited and reported inconclusive results. Provided that all the sex hormones originate from DHEA and convert to ASD then from ASD to T, oestrone sulphate or E2 and might either act directly or act after conversion; and SHBG is the transport protein for these hormones, all the sex hormones and SHBG should be examined in the same population. This thesis reported, lower E2 concentration was a risk factor for knee OA and that lower Ad concentration and greater SHBG concentration were risk factors for hip OA. E2 has a protecting role against the development of knee OA, as this hormone was found to preserve articular cartilage and subchondral bone (152). ASD is associated with increasing BMD at both the metaphyseal and diaphyseal regions of the femur (153, 154), and is responsible for increasing bone mineral quality and quantity in both

cancellous and cortical bone (155). SHBG binds E2 and ASD with high affinity (156), therefore higher concentrations of SHBG result in lower concentrations of unbound sex steroids (156). It may be that higher concentration of ASD and lower concentration of SHBG reduce the risk of hip OA and subsequent hip joint replacement through anabolic and anti-reabsorptive actions. In summary, these findings suggest that there is a role of these sex steroids in the pathogenesis of knee and hip OA and modifying them may provide potential strategies for the prevention and treatment of large joint OA.

Exposure to *in utero* higher parental testosterone and lower estrogen concentrations results in low 2D:4D (84-87). The 2D:4D ratio is always consistent among men and women with men having a lower average 2D:4D than women (84, 85). Sexual ability, facial shape, physical and athletic ability, performance in examinations, myocardial infarction has been linked to the lower 2D:4D (88). Through physical activity or through hormonal factors 2D:4D might be related to OA. However, previous studies have yielded inconclusive results regarding the relationship of 2D:4D and the risk of knee and hip OA. Moreover, till date no longitudinal study has explored the relationship between 2D:4D and the risk of knee and hip OA. This thesis demonstrated that, lower 2D:4D was associated with an increased risk of severe knee OA requiring knee joint replacement. There is no relationship of 2D:4D with risk of hip OA. These results may be explained in part by joint injuries associated with high-level physical activity in those with lower 2D:4D and the greater susceptibility of knee OA in response to injury than hip OA; they may also reflect hormonal influences on the growth of bone, cartilage and soft tissue.

7.3 Metabolic and vascular factors and risk of knee and hip osteoarthritis

The association between components of MetS, apart from central obesity and vascular disease with OA is not well understood. As age and obesity are the common risk factors for MetS, vascular disease and OA, it is unclear whether these conditions simply coexist or there is a causal relationship.

Previous studies showed inconsistent finding for the components of MetS and knee and hip OA. The baseline (93) and the 3 year follow-up of the ROAD study (34) showed that incidence and progression of radiographic knee OA was associated with MetS. However, they failed to adjust for obesity, which is the major driver for knee OA. The Malmö study explored the association between MetS components and the knee and hip OA, and found no association after adjustment for obesity (31). This thesis has demonstrated that after adjustment for BMI; central obesity, hypertension, and the MetS were significantly associated with knee OA. Also, with the accumulation of MetS components, the incidence of severe knee OA increased independent of BMI. In contrast, the incidence of severe hip OA was not related to either the individual or cumulative MetS components. The mechanism involved in the pathogenesis of knee OA through MetS is complex and is probably due to the change in the homeostasis between adipogenesis and osteogenesis and/or chondrogenesis (42). Individuals with MetS have an adverse metabolic profile including dyslipidemia (98, 99), which influence the production of adipocytokines (157) resulting in low grade inflammation (32, 158); hyperglycaemia (43) and insulin resistance (159); hypertension (97, 160); all of which may contribute to the initiation and progression of OA (108). It may also be that a common pathway is via vascular pathology and its effects on joint nutrition (144) which may trigger apoptosis of osteocytes and activate osteoclastic resorption and reduced

bony support for the overlying cartilage (161). These findings suggest that the management of the MetS may reduce the burden of knee OA.

Two previous studies have examined the risk of knee OA in relation to carotid artery atherosclerosis (106) and popliteal artery atherosclerosis (117), suggesting that macrovascular disease is involved in the pathogenesis of OA. The microcirculation, including the arterioles, capillaries, and venules, optimizes nutrient and oxygen supply within tissues and some of the earliest manifestation of cardiovascular disease occurs in the microcirculatory bed (143). There is a paucity of research exploring the relationship of microcirculation with risk of OA. This thesis found that narrower retinal arteriolar caliber, a unique way to assess the microcirculation, predicted an increased risk of knee replacement for OA, suggesting a role of microcirculation in the pathogenesis of knee OA. Articular cartilage is avascular and likewise depends on synovial fluid and subchondral bone for nutrition, for example supply of glucose, oxygen and water requirements. Articular cartilage also depends on subchondral bone for structural support (144). Synovium, a highly vascular tissue with arterioles, capillaries and venules, produces synovial fluid and is the main source of nutrition for articular cartilage (162). Likewise, subchondral bone is also a highly vascularised structure and depends on blood flow and haemopoiesis for its growth, repair and metabolism as well as modeling and remodeling (144). Narrower arteriolar calibre may result in impaired tissue perfusion in the synovium and subchondral bone, as consequences may induce localised hypoxia that stimulates angiogenesis, development of an immature vasculature and inflammation predictive of cartilage damage and catabolic effects on chondrocytes in OA (162). The ischaemic episodes in the subchondral bone lead to increased bone resorption and subsequent articular damage (144). Therefore it is likely that narrowing of arterioles impairs the integrity of subchondral bone and the supply of oxygen and nutrients to the overlying

cartilage plate, resulting in subsequent cartilage damage (144). Moreover, the knee depends on soft tissue and neuromuscular control for its stability. Arteriolar narrowing may also affect soft tissue integrity (163). Our findings support the notion that the microcirculation is involved in the pathogenesis of knee OA through arteriolar narrowing. Modification of microcirculation via lifestyle and pharmacological interventions may represent a potential target for the prevention and treatment of knee OA.

7.4 Serum 25(OH)D and risk of hip osteoarthritis

Vitamin D status might affect the development and progression of OA, especially hip OA, either directly via its effects on cartilage, or indirectly via its effects on bone (54). However, the findings from a number of epidemiological studies with regard to the risk of hip OA in relation to serum 25(OH)D concentrations are inconclusive, for example, some studies have reported that low serum 25(OH)D concentrations are associated with an increased prevalence and incidence of hip OA (54, 124), others have shown no effect (57, 58) of serum 25(OH)D on hip OA. Besides, many of these studies had a number of limitations which make interpretation of the findings difficult, including small sample size (54, 58, 124), cross-sectional study design (124), and failure to adjust for season of blood collection, which affects serum 25(OH)D concentrations significantly (54). In addition, studies have shown that vitamin D has a biphasic effect on bone mass: either low or high concentrations can potentially accelerate bone resorption (164, 165). This thesis showed, higher serum 25(OH)D concentrations was associated with an increased risk of hip OA requiring hip joint replacement in males but not in females, independent of age, BMI, smoking status, physical activity, season of blood collection and latitude, comorbidities and socioeconomic position. These results persisted even when those with low concentrations of 25(OH)D were excluded. Recent sequential meta-analysis findings on vitamin D supplementation with or without

calcium suggest that vitamin D does not reduce adverse skeletal outcomes in community-dwelling individuals (166). This study in the setting of widespread vitamin D supplementation adds to the ongoing debate about optimal serum concentrations of vitamin D for skeletal health.

7.5 Low birth weight and preterm birth and risk of knee and hip osteoarthritis

LBW and preterm birth more recently have been associated with reduced bone mass (64). As a potential mechanism, lack of osteoblastic differentiation of mesenchymal stem cells and new bone formation resulting in suppression of proper bone microarchitecture and mass has been suggested (67). Postural deformity (126) and unstable hip type IIc (risky) and IId (decentralized) (127) has been linked with an increased incidence of hip OA (167-169), is associated with low birth weight and preterm birth (90, 100). This thesis found LBW and preterm birth were associated with an increased risk of hip but not knee OA. The possible mechanism for this finding may be mediated through abnormal hip development as these babies are born early and the acetabulum is underdeveloped (151, 170). Preterm infants often develop a postural deformation of the legs which persists till early childhood (126), perhaps because of an underdeveloped or shallow, upwardly sloping acetabulum (170), decreased joint surface area(167), or because the ligaments holding the ball in place are too loose (126). There is evidence to support that preterm birth and very low birth weight results in decrease in bone formation and increase in bone resorption (171, 172), reduced osteoclast apoptosis (173) and cartilage degeneration (174). These factors may influence the development of the hip, resulting in abnormal hip joint shape. Another possible mechanism might be post-delivery postural change; the hips are extended rather than being maintained in a flexed and abducted in utero position (175, 176). This altered hip position may potentially be responsible for an increased incidence or severity of acetabular dysplasia. Probably modifying hip

position through postural support (175, 176) and perhaps the use of double nappies (177) may be beneficial for babies born with LBW or preterm, and they may need to be identified as "at risk group" and targeted for screening and early treatment of hip dysplasia.

7.6 Osteoarthritis of knee and hip: associated with different risk factors

There is increasing evidence suggesting that the risk factors for knee and hip OA are different. For example, there is sex specific difference that knee OA is prevalent in women over 50 years (18, 19), and hip OA more prevalent among men (3, 18). There are different genetic factors associated with knee and hip OA (37). Obesity (31), meta-inflammation produced by adipose cells (32), and metabolic factors (33, 34) are associated with knee OA, while hip dysplasia (35, 36), abnormal acetabular and femoral shape (35) are associated with the risk of hip OA. However, to date few studies have examined the risk of knee and hip OA in the same population (31). Examining the risk factors in the same population for both the knee and hip OA has the advantage of concluding the result without the influence of population variation.

This thesis has explored different risk factors for knee and hip OA in the same population. Taken together, these results suggest the consistent finding that, risk factors of severe knee and hip OA differs. Different susceptibility of knee and hip OA was observed for sex hormones; for example lower circulating concentration of E2 was associated with increased risk of knee OA; and lower circulating concentration of ASD and higher circulating concentration of SHBG was associated with hip OA. Lower 2D:4D were associated with increased risk of severe knee OA but not with hip OA. The risk of knee OA is more related to metabolic and vascular disorders such that, individual as well as cumulative components of MetS was linked to the risk of severe knee OA with no such relationship seen for severe hip

OA. Similarly, narrower retinal arteriolar caliber a marker of microcirculation was associated with increased risk of knee OA. LBW and preterm birth was associated with the risk of hip OA but not knee OA. Similarly higher circulating concentration of 25(OH)D was associated with increased risk of severe hip OA in males (Figure 2).

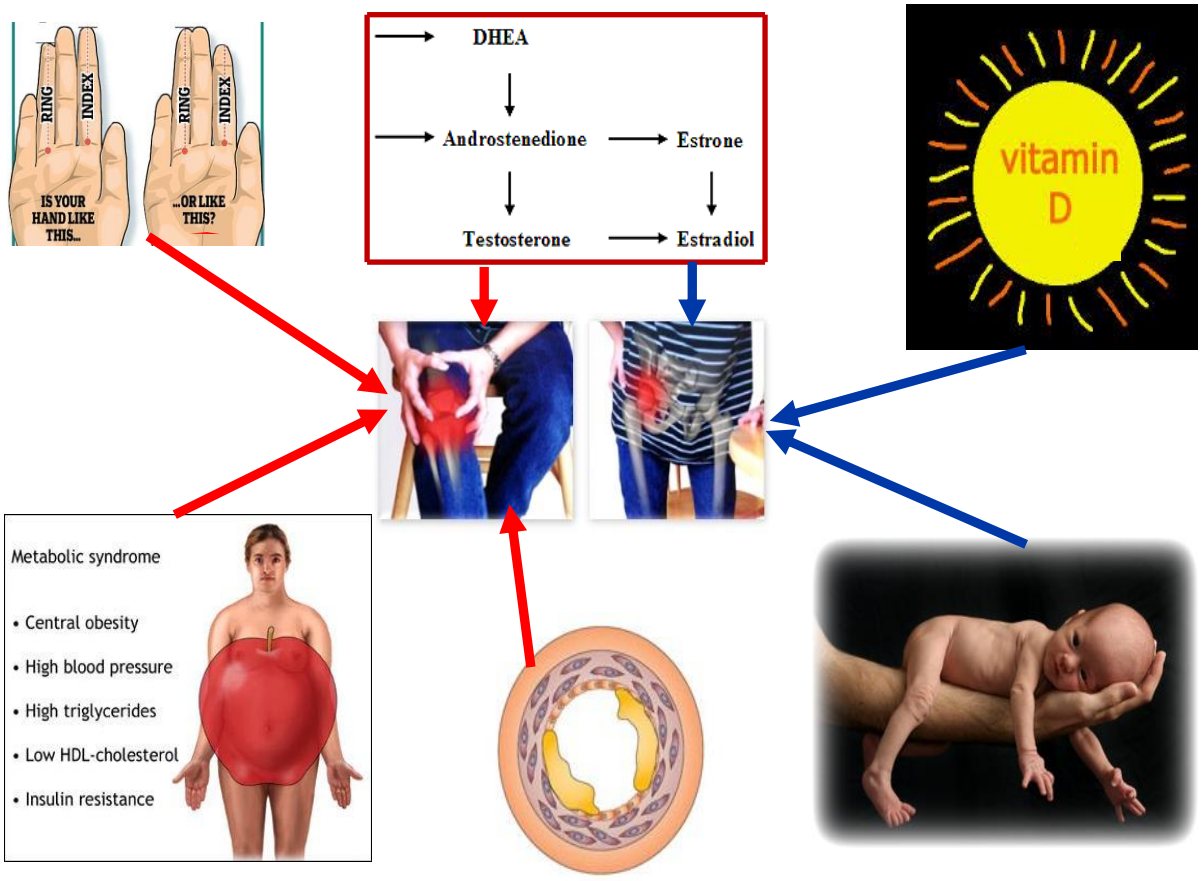


Figure 2: Risk factors for knee and hip OA

The differed findings for knee and hip OA in relation to circulating sex hormones, 2D:4D, MetS, LBW and preterm birth might be due to different susceptibility of these two joints to different risk factors. The knee is more dependent on soft tissue and neuromuscular control for its stability. Metabolic factors have been shown to affect soft tissue integrity. For example, MetS has been associated with intracellular fat deposition in muscle (178) and tendon structure (178, 179). E2 has greater influence on the soft tissue (69, 180-182) by

exerting direct and indirect effects on the maintenance and well-being of skeletal muscle and cartilage through ERα and ERβ via several pathways such as transforming growth factor β and the insulin-like growth factor I and II pathway (69, 180, 181). Furthermore, E2 plays an important role in fibroblast metabolism (182). The knee OA but not hip OA associated to 2D:4D may be explained, at least in part, by the different susceptibility of knee and hip OA in response to injury. The Johns Hopkins Precursors Study, revealed that early knee injuries were associated with almost a 3 (95% CI, 1.35, 6.45) times relative risk of symptomatic knee OA, with no association between hip injuries and later development of hip OA (183). It may also be that MetS as well as vascular disease affects joint nutrition (144). The effect of MetS on blood vessels over time may result in vessel narrowing (144, 145), reduced blood flow (144) and subchondral ischemia leading to cartilage degeneration due to reduced nutrient and gas supply from the subchondral bone (144, 184). There is evidence that arteriolar narrowing is associated with decreased perfusion of skeletal muscle, resulting in muscular atrophy (163) may also affect soft tissue integrity. In contrast, at the hip, bony shape and joint congruence appear to have a greater role in the development of hip OA (35), making the hip less susceptible to the effects of meta-inflammation. Furthermore, bony shape and joint congruence depend on osteoclast formation and bone resorption, which are likely to be affected by direct and indirect inhibitory effects of T (185). That lower ASD and higher SHBG are risk factors for hip OA is consistent with this androgen drive. In cultured osteoblast cells, ASD converts into T and dihydrotestosterone through the 17 β hydroxysteroid dehydrogenase pathway (186) which affects bone maturation and maintains the homeostasis of mature bone (186, 187). LBW and preterm birth have significant impacts on hip bone and structure. The formation of the acetabulum is incomplete at birth in preterm babies (151). Preterm infants often develop a postural deformation of the legs which persists till early childhood (126), as a result preterm birth is related to hip OA but not knee OA.

Similarly, the positive association between concentrations of serum 25(OH)D and hip bone mass and/or BMD (122) may explain the deleterious effect of higher serum 25(OH)D concentration in the pathogenesis of hip OA through changing shape and size of the bone (188). Our findings provide further support for the available evidence that differences exist between the pathogenesis of knee and hip OA.

To sum up, this thesis showed knee and hip OA has different susceptibility to different risk factors independent of population variation.

Chapter 8: Limitations and strengths

8.1 Limitations

8.1.1 Generalizability of study findings

All the work presented in this thesis examined whether a participant had knee or hip joint replacement for OA as a surrogate measure of symptomatic end-stage OA. Therefore, the conclusions from the thesis cannot be generalized to OA populations irrespective of OA severity. These findings may not be generalizable to people with radiographic OA.

8.1.2 Misclassification of outcome

There may be misclassification of the outcome measurement. For example, the MCCS study had collected baseline data between 1990 and 1994, and the AusDiab study between 1999 and 2000. The data linkage for joint replacement was available from 2001 for the MCCS and 2002 for the AusDiab study. Therefore there were no complete and reliable joint replacement data for the study populations prior to 2001/2002. As a result, some misclassification of joint replacement status may have occurred. This is likely to be non-differential in relation to the exposure measures, and thus may have biased the results towards the null. Joint replacements tend to be offered to patients who are otherwise relatively medically fit. The reluctance of orthopaedic surgeons to operate on patients who are obese or with obesity-related comorbidities would have resulted in an underestimation of the associations between exposures (MetS, retinal arteriole, sex hormones, 2D:4D, LBW and preterm birth, serum 25(OH)D concentration) and the risk of joint replacement for OA observed in this study.

8.1.3 Misclassification of exposure

The studies may have misclassification of exposure variables as well. For the circulating sex hormone and SHBH with risk of knee and hip OA, the blood samples were collected at various times throughout the day, so the circadian rhythms in hormone secretion may have been a source of variance (189). For the study examining the association between the components of MetS and risk of knee and hip OA, there was no record on lipid lowering and hypoglycaemic medications, which might have resulted in participants with naturally adverse serum lipid and glucose profiles being classified as having normal serum measures. The serum 25(OH)D and risk of hip OA study has the limitation of measuring serum 25(OH)D only at baseline, which may not reflect long term vitamin D status. Furthermore, no data was collected regarding vitamin D supplementation. These may have resulted in non-differential misclassification of exposures, which is likely to bias the results to the null.

The study regarding 2D:4D and risk of knee and hip OA had the limitations of using photo-copies of the hand that tend to yield lower 2D:4D values compared with direct measurement from hand radiographs (190). However, this measurement technique was used for all the participants. This would tend to result in non-differential misclassification of 2D:4D, which is not related to the outcome. Including participants whose fingers had features that might have affected the validity of the measurements in the analysis would have resulted in non-differential misclassification of 2D:4D categories, which may have underestimated the observed associations. Thus sensitivity analyses were performed by excluding these participants and similar results were obtained.

The study regarding LBW and preterm birth and the risk of knee and hip OA used self-reported birth weight and preterm birth data. Individuals with the highest and lowest birth

weights tend to report normal birth weight (191) which will lead to underestimation of joint replacement risk associated with LBW. Nevertheless, this study found a significant association of LBW and prematurity with hip but not knee joint replacement. It is unlikely that any misclassification would affect the relationship with joint replacement at the hip but not the knee.

8.1.4 Joint replacement as a surrogate measure of osteoarthritis

Defining OA based on joint replacement performed for OA only identify the tip of the iceberg of the very large OA population. Joint replacement as the treatment of OA may be influenced by a number of factors such as access to health care, physician bias, and patient-level factors, in addition to disease severity (192). We have adjusted for age, BMI, ethnicity, smoking status, physical activity, comorbidity and socioeconomic status to account for this issue.

8.1.5 Selection bias

The study regarding MetS and knee and hip OA, had serum lipid measurement for 68% of the study participants and only those were included in the analysis of cumulative components of MetS and MetS as a whole. There were differences between the attendees and non-attendees in relation to the distribution of age, sex, BMI, waist circumference, prevalence of hypertension, impaired fasting glucose, country of birth, education and physical activity which showed that the non-attendees had worse health condition. Fasting serum glucose results were not available for all participants at the same time when the serum lipids were measured, thus we used serum glucose at study inception. However, we compared the difference between fasting serum glucose levels at baseline and when lipid levels were taken

(for those for whom serum glucose was measured at both time points) and found no significant difference in serum glucose.

The study population for vascular factors (assessed by retinal vascular diameter) and risk of knee OA, was enriched for those most likely to have complications of diabetes compared with a control group (193), so is not directly generalizable to the general population. However, a subgroup analysis was done stratified by the diabetic status of the population, which revealed that the course of the disease was the same irrespective of diabetic status.

The study participants for the LBW and preterm birth and the risk of knee and hip OA, were younger, less likely to have diabetes and hypertension, and had lower BMI compared with individuals who did not respond to the birth weight and preterm birth questionnaire or could not recall their birth weight. However, when the risk of joint replacement of those who reported their birth weight versus those who did not was compared, there was no difference in risk of knee or hip joint replacement for OA (all $p > 0.80$).

8.1.6 Residual confounding

The observed findings in this thesis might be due to residual confounding. However, if residual confounding is the main explanation for the observed association between exposures (sex hormones, MetS, 2D:4D, LBW and preterm birth) and OA risk, the association would reflect similar direction for knee and hip joint replacement which was not the case for any of the study.

8.2 Strengths

8.2.1 Incidence of joint replacement ascertained by AOA NJRR

This thesis linked records from existing cohort studies to the AOA NJRR which collected validated and complete joint replacement data in Australia (140). AOA NJRR collect joint replacement data from both public and private hospitals and validate using a sequential multi-level matching process against State and Territory Health Department unit record data (140). Furthermore, using joint replacement to define OA has the advantage of recognizing severe OA, which has an unambiguous connection with the disease burden.

8.2.2 Large cohort studies via data linkage

All the studies were prospective in nature as a result it provided a unique opportunity to establish a causal relationship. All the studies used large sample size, participants from different ethnic backgrounds which enriched this thesis by looking at ethnic variations. This thesis ascertained the incidence of knee and hip joint replacement by linking the data between AOA NJRR, and MCCS and AusDiab study which have had the data on potential risk factors collected before the outcomes of interest occurred. This is a cost effective way to get quality evidence of a disease outcome.

8.2.3 Examination of knee and hip osteoarthritis in the same population

Most of the studies included in this thesis have examined factors associated with the risk of knee and hip OA in the same population and have demonstrated the mechanism of the disease differs at these joints. This is important to eliminate the confounding effect related to population variation.

8.2.4 Measurement of exposures

In measurement of exposure this thesis has several strengths; for example for the study looking at circulating sex hormone and risk of knee and hip OA, we excluded women who were using any exogenous hormone therapy at baseline. Those who might use exogenous hormone during the course of follow-up time did not bias the findings reported in this study because the participants were asked about exogenous steroid therapy during first round and the second round of MCCS follow-up in 1995-1998 and 2003-2007, with negligible numbers of women (n=31 out of 2366, and n=4 out of 1860, respectively) reporting exogenous steroid therapy. The results did not vary substantially after excluding these individuals from the analysis.

For the retinal vascular caliber and risk of knee OA, the retinal vascular calibre was measured using a nonmydriatic digital fundus camera for retinal photography of both eyes and use of a validated computer software program to measure retinal vascular caliber (194).

8.2.5 Adjustment for potential confounders

In this thesis all the studies were adjusted for the known confounders of OA including age, sex, BMI, ethnicity, physical activity, factors associated with socioeconomic status. Furthermore, special consideration was given to study specific exposure and outcome and the associated confounders for adjustment. For example, the retinal vascular caliber and risk of knee OA was further adjusted for HbA1c, systolic blood pressure, total cholesterol and microalbuminuria; the LBW and preterm birth with risk of knee and hip OA for hypertension, diabetes and smoking, the concentration of serum 25(OH)D and risk of hip OA for smoking status, season of blood collection and latitude, hypertension and diabetes.

Chapter 9: Future directions

Although this thesis has examined a number of concepts, a general theme has emerged and that is OA of the knee and hip have systemic (sex hormones, MetS) predispositions which acts with the local (mechanical) factors; there are novel risk factors for hip OA [LBW and preterm birth, 25(OH)D]; and that knee and hip OA are susceptible to different risk factors.

All the works included in this thesis have defined knee and hip OA as having a joint replacement for knee OA or hip OA which can only identify people with established severe disease. As a result it is hard to describe whether these risk factors are associated with either initiation or progression or with both. While some of the current work suggests that OA initiation is separate than the disease process (195, 196), therefore future studies should attempt to ensure the cohorts are disease free or at least the stage of the disease at the beginning.

For the circulating sex hormones and risk of knee and hip OA, our study was the first study of its kind to explore the association of the all the sex hormones and knee and hip OA, provided the complexity of sex hormones and risk of OA further research are needed to confirm these results. If the results are proven, randomized clinical trials (RCT) with E2 supplementation to prevent knee OA and ASD supplementation to prevent hip OA should be designed. This thesis has measured adult 2D:4D and explored the relationship with hip and knee OA. Further study should be done to check whether childhood finger ratio correlates with our findings.

While components of MetS and cumulative MetS; and microcirculatory disease has been linked to knee OA, further work is required to help better understand how these risk factors are contributing to the initiation and/or progression of OA. Whether targeting one component or all the components as a whole will benefit targeting the disease need to be explored. For example, inflammation is common for both MetS and microcirculatory disease. Probably targeting OA with anti-inflammatory agents might benefit the disease. Nonetheless, RCTs through managing MetS and microcirculation by lifestyle modification and/or through pharmacological treatment warrant consideration for the prevention and treatment of OA.

A relationship between the baseline higher serum concentration of 25(OH)D was found as a risk factor for hip OA over an average 9.1 (SD 2.7) years of follow-up. The fact that serum 25(OH)D concentration was measured only one time in the study included in this thesis, may be participants have taken some vitamin D medication or their 25(OH)D concentration has been changed overtime which was not addressed. Further study should be done taking this matter into account.

There is a relationship between LBW and preterm birth with postural deformity (126); unstable hip type IIc (risky) and IId (decentralized) (127); and reduced bone mass (64); which are linked with an increased incidence of hip OA (167-169). Prior to our work no studies have examined the association between LBW and preterm birth and hip OA. In our study birth weight and history of preterm birth are self-reported. To confirm this association, observational studies can be done where birth weight and preterm birth should be obtained from the hospital records. Nonetheless, LBW and preterm babies should be identified as an "at risk group" and should be monitored for early detection of hip OA.

Finally, the data examined in this thesis has focused on people with severe knee and hip OA. It is possible that established OA responds differently to hormones, MetS and vascular disease factors whose effects may be mediated through associated with bone changes in bone shape; may be in the progression of the disease. Since OA initiation and progression depend on different risk factors (195, 196), repeating these studies in a cohort of healthy population without clinical OA would help to understand whether hormones, MetS and vascular disease, serum 25(OH)D, and LBW and preterm birth influence the initiation of disease.

Chapter 10: Conclusion

In the first part of this thesis, the association between the circulating sex hormones, and 2D:4D ratio, and risk of severe knee and hip OA was explored. It was shown that, lower E2 concentration is a risk factor for knee OA requiring knee replacement and lower ASD concentration and greater SHBG concentration are risk factors for hip OA requiring hip replacement in women. Similarly, lower 2D:4D was associated with an increased incidence of knee OA requiring knee replacement.

The second part examined metabolic and vascular factors and the risk of knee and hip replacement for OA. It has established that the MetS and all its components were associated with increased incidence of severe knee OA. Even after adjustment for BMI, the associations of central obesity, hypertension, and the MetS remained significant. With the accumulation of MetS components, the incidence of severe knee OA increased independent of obesity. For the vascular disease and risk of severe OA, this thesis found that narrower retinal arteriolar calibre, a unique way to assess the microcirculation predicted an increased risk of knee replacement.

The third part examined whether the circulating 25(OH)D concentration was associated with the risk of severe knee and hip OA. It was found that higher serum 25(OH)D concentration was a risk factor for severe hip OA in males but not in females.

Finally, this thesis examined whether LBW and preterm birth were associated with the risk of severe knee and hip OA. It was found that, LBW and preterm birth are linked to severe hip OA requiring hip joint replacement.

Taken together these results suggest that knee and hip OA are susceptible to different risk factors; such that lower circulating concentration of E2 and lower 2D:4D, and individual as well as cumulative components of MetS, microcirculation assessed by retinal arteriolar caliber, were associated with increased risk of severe knee OA. Likewise, lower circulating concentration of ASD and higher circulating concentration of SHBG, LBW and preterm birth, and higher circulating concentration of 25(OH)D was associated with increased risk of hip OA. This thesis has also demonstrated that certain people can be identified even before the disease onset and be targeted for prevention of OA, for example – women with lower 2D:4D can be identified for knee OA and babies born with LBW and preterm for hip OA. Finally people with knee and hip OA should be identified and their treatment modalities should be different; i.e. targeting E2 concentration along with MetS and vascular pathway will benefit the knee OA people; and maintaining a normal level of ASD, targeting factors associated with changes of bone shape will benefit the people with hip OA.

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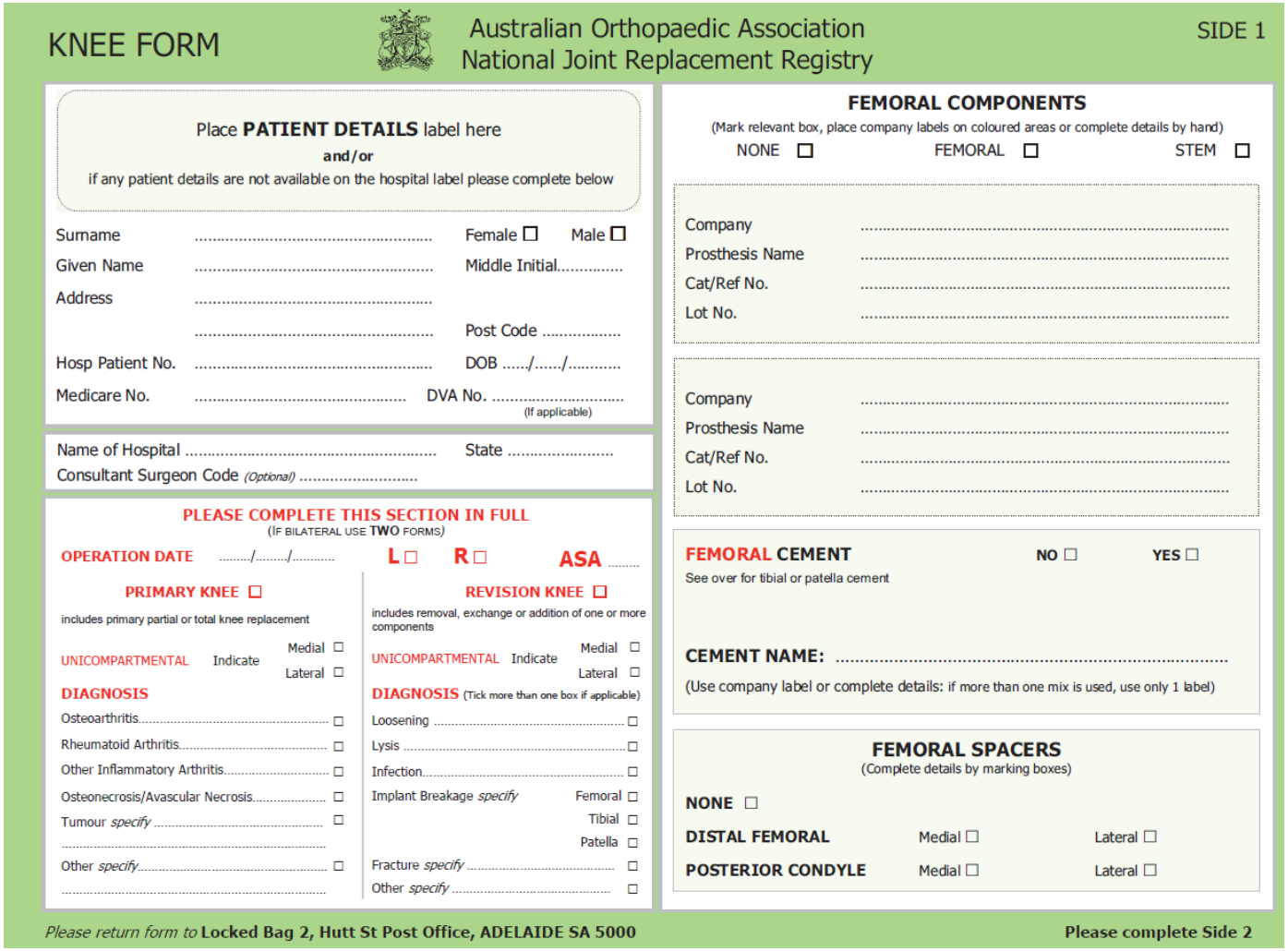
Appendix

Appendix 1: Data collection form for knee and hip replacement



KNEE FORM

Australian Orthopaedic Association
National Joint Replacement Registry

SIDE 1

Place **PATIENT DETAILS** label here
and/or
if any patient details are not available on the hospital label please complete below

Surname ..... Female ☐ Male ☐
Given Name ..... Middle Initial.....
Address .....
..... Post Code .....
Hosp Patient No. ..... DOB ...../...../.....
Medicare No. ..... DVA No. .....
(If applicable)

Name of Hospital ..... State .....
Consultant Surgeon Code *(Optional)* .....

PLEASE COMPLETE THIS SECTION IN FULL
(IF BILATERAL USE **TWO** FORMS)

OPERATION DATE ...../...../..... **L** ☐ **R** ☐ **ASA** .....

PRIMARY KNEE ☐
includes primary partial or total knee replacement

UNICOMPARTMENTAL Indicate Medial ☐ Lateral ☐

DIAGNOSIS
Osteoarthritis ☐
Rheumatoid Arthritis ☐
Other Inflammatory Arthritis ☐
Osteonecrosis/Avascular Necrosis ☐
Tumour *specify* ☐
.....
Other *specify* ☐
.....

REVISION KNEE ☐
includes removal, exchange or addition of one or more components

UNICOMPARTMENTAL Indicate Medial ☐ Lateral ☐

DIAGNOSIS (Tick more than one box if applicable)
Loosening ☐
Lysis ☐
Infection ☐
Implant Breakage *specify* Femoral ☐ Tibial ☐ Patella ☐
Fracture *specify* ☐
Other *specify* ☐

FEMORAL COMPONENTS
(Mark relevant box, place company labels on coloured areas or complete details by hand)
NONE ☐ FEMORAL ☐ STEM ☐

Company .....
Prosthesis Name .....
Cat/Ref No. .....
Lot No. .....

Company .....
Prosthesis Name .....
Cat/Ref No. .....
Lot No. .....

FEMORAL CEMENT NO ☐ YES ☐
See over for tibial or patella cement

CEMENT NAME: .....
(Use company label or complete details: if more than one mix is used, use only 1 label)

FEMORAL SPACERS
(Complete details by marking boxes)
NONE ☐
DISTAL FEMORAL Medial ☐ Lateral ☐
POSTERIOR CONDYLE Medial ☐ Lateral ☐

Please return form to **Locked Bag 2, Hutt St Post Office, ADELAIDE SA 5000**

Please complete Side 2

KNEE FORM

Australian Orthopaedic Association
National Joint Replacement Registry

SIDE 2

TIBIAL COMPONENTS

(Mark relevant box, place company labels on coloured areas or complete details by hand)

NONE ☐ ALL-IN-ONE ☐ BASE PLATE ☐ INSERT ☐ STEM ☐

Company .....
Prosthesis Name .....
Cat/Ref No. .....
Lot No. .....

Company .....
Prosthesis Name .....
Cat/Ref No. .....
Lot No. .....

Company .....
Prosthesis Name .....
Cat/Ref No. .....
Lot No. .....

TIBIAL CEMENT NO ☐ YES ☐

CEMENT NAME: .....

(Use company label or complete details: if more than one mix is used, use only 1 label)

TIBIAL SPACERS

(Complete details by marking boxes)

NONE ☐ **BLOCKS** Medial ☐ Lateral ☐

WEDGES Medial ☐ Lateral ☐

SCREWS **NO** ☐ **YES** ☐ Number .....

PATELLA COMPONENT

(Mark relevant box, place company labels on coloured areas or complete details by hand)

NONE ☐ **YES** ☐

Company .....
Prosthesis Name .....
Cat/Ref No. .....
Lot No. .....

PATELLA CEMENT NO ☐ YES ☐

CEMENT NAME: .....

(Use company label or complete details: if more than one mix is used, use only 1 label)

COMPUTER ASSISTED NO ☐ YES ☐

System used: .....
.....

ADDITIONAL COMMENTS (or Extra Labels)

ALL SECTIONS of this form MUST be COMPLETED

Thank you for completing this form - For further information contact (08) 8313 3592

Completed by ..... **Date** ...../...../.....

HIP FORM

Australian Orthopaedic Association
National Joint Replacement Registry

SIDE 1

Place **PATIENT DETAILS** label here

and/or

if any patient details are not available on the hospital label please complete below

Surname ..... Female ☐ Male ☐

Given Name ..... Middle Initial.....

Address .....

..... Post Code .....

Hosp Patient No. ..... DOB ...../...../.....

Medicare No. ..... DVA No. .....
(If applicable)

Name of Hospital ..... State .....

Consultant Surgeon Code *(Optional)* .....

PLEASE COMPLETE THIS SECTION IN FULL
(IF BILATERAL USE TWO FORMS)

OPERATION DATE ...../...../..... **L** ☐ **R** ☐ **ASA** .....

PRIMARY HIP ☐

includes Unipolar (Austin Moore or Thompson etc), Bipolar or Total Hip Replacement

DIAGNOSIS

Osteoarthritis ..... ☐
Rheumatoid Arthritis ..... ☐
Other Inflammatory Arthritis..... ☐
Osteonecrosis/Avascular Necrosis ..... ☐
Developmental Dysplasia..... ☐
Fractured Neck of Femur..... ☐
Tumour *specify*..... ☐
.....
Other *specify*..... ☐
.....

REVISION HIP ☐

includes removal, exchange or addition of one or more components

DIAGNOSIS (Tick more than one box if applicable)

Loosening ..... ☐
Lysis ..... ☐
Dislocation..... ☐
Infection..... ☐
Implant Breakage Stem ..... ☐
Acetabular ..... ☐
Fracture *specify* ..... ☐
.....
Other *specify* ..... ☐
.....

ACETABULAR COMPONENTS

(Mark relevant box/es, place company labels on coloured areas or complete details by hand)

NONE ☐ CUP ☐ SHELL ☐ INSERT ☐ BIPOLAR ☐ REINFORCEMENT RING ☐ MESH ☐

Company .....
Prosthesis Name .....
Cat/Ref No. .....
Lot No. .....

Company .....
Prosthesis Name .....
Cat/Ref No. .....
Lot No. .....

Company .....
Prosthesis Name .....
Cat/Ref No. .....
Lot No. .....

ACETABULAR CEMENT **NO** ☐ **YES** ☐

See over for femoral cement

CEMENT NAME: .....

(Use company label or complete details: if more than one mix is used, use only 1 label)

(Complete by hand, labels not required)

SCREWS: **NO** ☐ **YES** ☐ **Number used** .....

Please return form to **Locked Bag 2, Hutt St Post Office, ADELAIDE SA 5000**

HIP FORM

Australian Orthopaedic Association
National Joint Replacement Registry

SIDE 2

FEMORAL COMPONENTS

(Mark relevant box/es, place company labels on coloured areas or complete details by hand)

NONE ☐ STEM ☐ HEAD ☐ CENTRALISER ☐ INTRAMEDULLARY PLUG ☐

Company .....
Prosthesis Name .....
Cat/Ref No. .....
Lot No. .....

Company .....
Prosthesis Name .....
Cat/Ref No. .....
Lot No. .....

Company .....
Prosthesis Name .....
Cat/Ref No. .....
Lot No. .....

Company .....
Prosthesis Name .....
Cat/Ref No. .....
Lot No. .....

Company .....
Prosthesis Name .....
Cat/Ref No. .....
Lot No. .....

FEMORAL CEMENT NO ☐ YES ☐

See over for acetabular cement

CEMENT NAME: .....

(Use company label or complete details: if more than one mix is used, use only 1 label)

ADDITIONS

(Use company label for grip and cable and/or complete details)

TROCHANTERIC GRIP: NO ☐ YES ☐

Company: .....

CABLE/S: (For multiple cables use 1 label) NO ☐ YES ☐

Number used: ..... **Company:** .....

WIRE: (Complete by hand) ..... NO ☐ YES ☐

COMPUTER ASSISTED NO ☐ YES ☐

System used: .....
.....

ADDITIONAL COMMENTS (or Extra Labels)

ALL SECTIONS of this form MUST be COMPLETED

Thank you for completing this form - For further information contact (08) 8313 3592

Completed by ..... **Date** ...../...../.....

Appendix 2: Publications during PhD but not included in this thesis

1. **Hussain SM**, Cicuttini FM, Bell RJ, Robinson PJ, Davis SR, Giles GG, et al. Reply: Comment on Incidence of total knee and hip replacement for osteoarthritis in relation to circulating sex steroid hormone concentrations in women. Arthritis & rheumatology (Hoboken, NJ). 2014.
2. **Hussain SM**, Oldenburg B, Wang Y, Zoungas S, Tonkin AM. Assessment of Cardiovascular Disease Risk in South Asian Populations. Int J Vasc Med. 2013;2013:786801.
3. **Hussain SM**, Boonshuyar C, Ekram ARMS. Non-adherence antihypertensive treatment is associated with family support in Bangladesh. Medimond International Proceedings 2012; 215-9.

Amendments

On Page vii paragraph 1 and Page 5 paragraph 1, the word "*currently"* has been added after registered disease modifying drug for clarity.

On Page vii paragraph 1, 'mechanisms underlying risk factors' have been amended as "*how risk factors lead to initiation and progression of OA"* for clarity.

On page 3 paragraph 2, 'the' has been added for grammatical correction "*the increase in life expectancy"*

On Page 4 paragraph 1, 'a comma' has been added for grammatical correction *"pain, which generally"*

On Page 15 paragraph 1, the following line has been added

"while the work by Barker centred around low birth weight as a manifestation of adverse intrauterine environment, subsequent studies considered the consequences of low birth weight as a manifestation of significant prematurity"

Reference for this line

1. Skilton MR, Viikari JS, Juonala M, Laitinen T, Lehtimaki T, Taittonen L, et al. Fetal growth and preterm birth influence cardiovascular risk factors and arterial health in young adults: the Cardiovascular Risk in Young Finns Study. Arterioscler Thromb Vasc Biol. 2011;31(12):2975-81.
2. Kistner A, Jacobson L, Jacobson SH, Svensson E, Hellstrom A. Low gestational age associated with abnormal retinal vascularization and increased blood pressure in adult women. Pediatr Res. 2002;51(6):675-80.

On page 15 the following table has been added to summarize the findings

Table 1. Risk factors currently known for knee and hip OA

Risk factors	What's known
Hormonal factors	1. There are gender differences in the prevalence, incidence and severity of knee and hip OA, especially knee OA affecting more women than men (38). The incidence and severity of OA has been reported to increase particularly after the menopause (38). Though several sex hormones, especially the effect of estrogen concentrations have been examined in a number of studies, no clear causal relationship has yet been established (39). There is no consistent evidence linking estrogen supplementation and risk of OA, as some studies showed beneficial effects of higher level of sex steroids (69, 70, 76, 77) while others have found no association (71, 72, 80), or even an increased risk (78, 79). 2. The ratio of the length of the index (2D) and ring (4D) fingers (expressed as 2D:4D) reflects the effects of prenatal sex steroids on 19 skeletogenic genes (82) and skeletogenic SMOC1 gene (83), suggesting a possible underlying genetic determinant of 2D:4D. A number of phenomena for example sexual ability, facial shape, physical and athletic ability, performance in examinations, myocardial infarction have been linked to the reduction of 2D:4D (88). OA can be related to 2D:4D through physical activity or through hormonal factors.

Risk factors	What's known
Metabolic and vascular factors	1. MetS and OA shares some common risk factors i.e. age and obesity. Though biomechanical loading exerted by obesity is one of the important aspects in the OA pathogenesis, recent advances in the physiology of adipose tissue add further insights in understanding the relationship between inflammatory and metabolic aspect of obesity and OA (32). Hypertension is more prevalent among the individuals with knee OA (31, 34, 92, 93), and hip OA (31); and increased serum cholesterol is more prevalent among the individuals of Knee OA (97-99) compared to non-OA individuals in observational studies. Among the participants of NHANES III survey, the individual components of the MetS were more prevalent in people with knee OA (92). Furthermore, MetS was found to be 5.26 times more common in participants with knee OA (92). 2. Increased intima media thickness of the carotid artery and increased popliteal artery wall thickness, subclinical markers of large vessel atherosclerosis, has been shown to be associated with increased prevalence of OA (106, 117), establishes the fact that macrovascular disease is involved in the pathogenesis of OA. However, little work has examined the role of microcirculation in the pathogenesis of OA.
Serum 25-hydroxy-vitamin D concentrations	1. Despite age, gender, obesity growing evidence suggests a role of the bone microenvironment, including osteoblast differentiation, the process of mineralization, and bone remodeling leading to change in the shape of the bone which is linked to hip OA (118). Serum 25(OH)D has been linked to changing the bone microstructure; bone mass and/or BMD (122), which can change the shape and geometry of the bone (46). Provided that hip is a bony joint, bony deformity related to change in bone shape will be more pronounced to hip OA.
Birth weight	1. Several adulthood diseases have their deep rooted link to the in utero programming. For example hypertension, insulin resistance, cardiovascular disease (63), and more recently reduced bone mass (64) have been linked to LBW and preterm birth. Preterm babies are found to develop a postural deformation of the legs with wide hip abduction and external rotation giving a 'frog leg' posture that persists till the age of 3-4.5 years (126). They also have high frequency of unstable hip type IIc (risky) and IId (decentralized) (127).

On Page 17 paragraph 3, the following line has been included to make the reader aware that there may be factors that impact on uptake of joint replacement

"However, undergoing a joint replacement as the treatment of OA may be influenced by a number of factors such as access to health care, physician bias, and patient level factors, in addition to disease severity".

On page 19 paragraph 1, *and Scotland* has been added