Childhood and Adulthood Determinants of Knee Joint Health in Young Adults

by

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(Medical Research)

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Statement of Originality

This thesis contains no material which has been accepted for a degree or diploma by the University or any other institution, except by way of background information and duly acknowledged in the thesis, and to the best of my knowledge and belief no material previously published or written by another person except where due acknowledgement is made in the text of the thesis, nor does the thesis contain any material that infringes copyright.

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Statement of Co-Authorship

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

Chapters 4

Antony B, Jones G, Blizzard L, Venn A, Cicuttini F, March L, Dwyer T, Marita C, Ding C. Childhood physical fitness predicts adulthood knee cartilage volume and bone area: a 25 year cohort study. Arthritis Care & Research 2015;18/03/15:Epub.

The contribution of each author:

BA was responsible for data management and cleaning, carried out analysis and interpretation of data, prepared the initial manuscript draft, and completed manuscript revisions.

AV, TD, LM and FC designed and carried out the study planning, and critically revised the manuscript.

LB participated in analysis and interpretation of data, and critically revised the manuscript.

MC participated in collection of data, and critically revised the manuscript.

GJ designed and carried out the study planning, participated the interpretation of the data, and critically revised the manuscript.

CD designed and carried out the study planning, participated in analysis and interpretation of the data, assisted with the initial manuscript draft, and critically revised the manuscript.

Chapter 5

Antony B, Jones G, Blizzard L, Venn A, Cicuttini F, March L, Dwyer T, Marita C, Ding C. Association between childhood overweight measures and adulthood knee pain, stiffness and dysfunction: a 25-year cohort study. Annals of the Rheumatic Diseases 2015; 74: 711-717

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GJ designed and carried out the study planning, participated the interpretation of the data, and critically revised the manuscript.

CD designed and carried out the study planning, participated in analysis and interpretation of the data, assisted with the initial manuscript draft, and critically revised the manuscript.

Chapter 6

Antony B, Venn A, Cicuttini F, March L, Blizzard L, Dwyer T, Marita C, Jones G, Ding C. Body composition, hormonal and inflammatory factors are associated with tibial cartilage volume in young adults and explain sex difference in cartilage volume. Arthritis Care & Research 2015;(In press).

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Chapter 7

Antony B, Venn A, Cicuttini F, March L, Blizzard L, Dwyer T, Marita C, Jones G, Ding C. Association of physical activity and physical performance with tibial cartilage volume and bone area in young adults. Arthritis Research & Therapy 2015;(Accepted for publication).

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AV, TD, LM and FC designed and carried out the study planning, and critically revised the manuscript.

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MC participated in collection of data, and critically revised the manuscript.

GJ designed and carried out the study planning, participated the interpretation of the data, and critically revised the manuscript.

CD designed and carried out the study planning, participated in analysis and interpretation of the data, assisted with the initial manuscript draft, and critically revised the manuscript.

Chapter 8

Antony B, Venn A, Cicuttini F, March L, Blizzard L, Dwyer T, Marita C, Jones G, Ding C. Correlates of knee bone marrow lesions in younger adults. Osteoarthritis and Cartilage 2015;(Under revision).

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Statement of Ethical Conduct

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

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Abstract

Osteoarthritis (OA) is the most common joint disorder in adults around the world. Knee OA is the most common form of OA and results in deterioration of knee structure and function for which there is no cure. The risk factors for OA include age, female sex obesity and injury, but the effect of physical activity on knee joint health is still controversial. Identifying modifiable risk factors early in life has the potential to prevent the development of knee OA in later life; however, there is sparse evidence relating childhood factors such as physical fitness and fatness to adult joint health. Knee magnetic resonance imaging (MRI) has revolutionised the knee OA research with its ability to quantify the different knee structures and to grade the abnormalities of these structures. Some of these quantified structures such as tibial bone area and tibial cartilage volume and the structural abnormalities such as subchondral bone marrow lesions (BMLs), cartilage defects and meniscal lesions are known to be associated with the development and progression of OA in later life.

The aims of this thesis are to determine whether physical activity, physical performance measures and fatness in childhood measured 25 years prior are associated with knee joint structure and symptoms in young adults, and to explore the correlates of knee structural and functional measures in young adults.

Participants broadly representative of the Australian population were selected from the Australian Schools Health and Fitness Survey (ASHFS) of 1985. Participants of ASHFS underwent anthropometric and physical performance measurements during childhood (age 7-15 years). They were followed up and these measurements were repeated 20 years later in Childhood Determinants of Adult Health (CDAH) study. Physical performance measures included physical fitness measures such as physical work capacity at 170 beats/min (PWC_{170}), running times, sit-ups, long jump and leg muscle strength. Physical activity, smoking and alcohol history were recorded using questionnaires. Body mass index (BMI), lean mass, fat mass and waist hip ratio were calculated from the anthropometric measurements.

A representative sub-sample (n=330, aged 31-41 years, female 47%) underwent magnetic resonance imaging (MRI) of their knees as well as questionnaires on knee pain five years later in CDAH Knee Cartilage study. MRI scans were processed to determine the structural measures such as tibial bone size and cartilage volume and structural abnormalities such as BML, cartilage defects, and meniscal abnormalities. The associations of childhood factors with adult knee structures and symptoms were

determined. The associations of adulthood factors such as physical activity, cholesterol levels, sex hormones and systemic inflammatory markers with adult knee structures were also determined.

Childhood physical performance measures including cardiorespiratory fitness measures were beneficially associated with adulthood knee bone size and cartilage volume, independent of the adult-attained fitness level. Cartilage volume accrual was partially mediated by bone size, indicating the importance of subchondral bone in the development of cartilage in adulthood.

Childhood overweight measures were associated with higher knee pain in adulthood independent of adulthood overweight status. The trajectory of overweight status from childhood to adulthood indicated that participants who were overweight at both childhood and adulthood have the highest prevalence and risk of knee pain in adulthood.

Knee cartilage volume measured in young adults was associated with body composition, physical activity, physical performance measures, sex hormones and fibrinogen measured five years prior. The association of physical activity and fitness with cartilage volume was independent of each other suggesting the influence of environmental factors and the potential for intervention to increase knee cartilage volume.

Subchondral bone abnormalities such as BMLs in young adults were positively associated with knee symptoms and other knee structural abnormalities such as cartilage defects and meniscal lesions. Moderate physical activity and higher high-density lipoprotein (HDL) cholesterol may be protective while vigorous physical activity may weakly increase the risk of medial tibiofemoral BMLs in young adults.

In conclusion, physical performance measures and/or physical activity in childhood and adulthood are beneficially associated with tibial cartilage volume and tibial bone size measured in adulthood. Obesity measures in childhood and adulthood are detrimentally associated with knee pain and/or structural abnormalities measured through MRI. These findings suggest the importance of increasing physical activity/fitness and reducing overweight in childhood and adulthood to have a healthy knee joint which may prevent the development of OA in later life. The positive association of sex hormone binding globulin and the negative association of inflammatory markers with knee cartilage volume indicate the possibility of intervention in modifying knee joint health.

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Publications Arising from the Thesis

Chapter 4: Antony B, Jones G, Blizzard L, Venn A, Cicuttini F, March L, Dwyer T, Marita C, Ding C. Childhood physical fitness predicts adulthood knee cartilage volume and bone area: a 25 year cohort study. Arthritis Care & Research 2015; 18/03/15: Epub.

Chapter 5: Antony B, Jones G, Blizzard L, Venn A, Cicuttini F, March L, Dwyer T, Marita C, Ding C. Association between childhood overweight measures and adulthood knee pain, stiffness and dysfunction: a 25-year cohort study. Annals of the Rheumatic Diseases 2015; 74: 711-717

Chapter 6: Antony B, Venn A, Cicuttini F, March L, Blizzard L, Dwyer T, Marita C, Jones G, Ding C. Body composition, hormonal and inflammatory factors are associated with tibial cartilage volume in young adults and explain sex difference in cartilage volume. Arthritis Care & Research 2015; (In press).

Chapter 7: Antony B, Venn A, Cicuttini F, March L, Blizzard L, Dwyer T, Marita C, Jones G, Ding C. Association of physical activity and physical performance with tibial cartilage volume and bone area in young adults. Arthritis Research & Therapy 2015;(Accepted for publication).

Chapter 8: Antony B, Venn A, Cicuttini F, March L, Blizzard L, Dwyer T, Marita C, Jones G, Ding C. Correlates of knee bone marrow lesions in younger adults. Seminars in Arthritis and Rheumatism 2015;(Under revision).

Other Publications During PhD

Antony B, Ding C, Stannus O, Cicuttini F, Jones G. Association of baseline knee bone size, cartilage volume, and body mass index with knee cartilage loss over time: a longitudinal study in younger or middle-aged adults. Journal of Rheumatology 2011; 38:1973-80

Sucharita S, Thomas T, **Antony B**, Vaz M. Vitamin B12 supplementation improves heart rate variability in healthy elderly Indian subjects. Autonomic Neuroscience 2012; 168:66-71

Antony B, Jones G, Stannus O, Blizzard L, Ding C. Body fat predicts an increase and limb muscle strength predicts a decrease in leptin in older adults over 2.6 years. Clinical Endocrinology (Oxf) 2013; 79:652-60

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Han W, Cai S, Liu Z, Jin X, Wang X, **Antony B**, Cao Y, Aitken D, Cicuttini F, Jones G, Ding C. Infrapatellar fat pad in the knee: is local fat good or bad for knee osteoarthritis? Arthritis Research & Therapy 2014; 16:R145

Pan F, Han W, Wang X, Liu Z, Jin X, **Antony B**, Cicuttini F, Jones G, Ding C. A longitudinal study of the association between infrapatellar fat pad maximal area and changes in knee symptoms and structure in older adults. Annals of the Rheumatic Diseases 2014; Epub

Stannus OP, Cao Y, **Antony B**, Blizzard L, Cicuttini F, Jones G, Ding C. Cross-sectional and longitudinal associations between circulating leptin and knee cartilage thickness in older adults. Annals of the Rheumatic Diseases 2015; 74:82-8

Antony B, Wang J, Han W, Wang X, Pan F, Liu Z, Jin X, Cicuttini F, Jones G, Ding C. Patellar bone marrow lesions predict patellar cartilage defect progression, cartilage volume loss and knee pain in older adults: a cohort study. Accepted in Osteoarthritis & Cartilage 2015; S1063-4584: Epub

Scientific Presentations During PhD

International

2012 American College of Rheumatology (ACR) Annual Conference 2012, Washington DC, USA. "Childhood physical fitness predicts adulthood knee cartilage volume and bone area" (Oral presentation)

2013 Annual European Congress of Rheumatology EULAR 2013, Madrid, Spain. "The association between childhood obesity measures and adulthood knee pain, stiffness and physical dysfunction" (Oral presentation)

2014 The American Society for Bone and Mineral Research (ASBMR) Conference 2014, Houston, USA. "Correlates of heel bone mass in young Adults: the role of cholesterol over 20 years from childhood to early adulthood" (Poster presentation)

2014 American College of Rheumatology (ACR) Annual Conference 2014, Boston, USA. 1) "Correlates of knee bone marrow lesions in younger adults"

2) "Physical performance and obesity measures are associated with tibial cartilage volume and explains the sex difference in cartilage volume" (Poster presentations)

2015 Osteoarthritis Research Society International (OARSI) Congress 2015, Seattle, USA. "Disruptive pathology rather than degenerative or discrete tear are associated with knee pain, increasing bone marrow lesion volume and a proxy for total knee arthroplasty: longitudinal analysis from the osteoarthritis initiative" (Poster presentation)

National

2013 Australian Rheumatology Association (ARA) Conference 2013, Perth, Australia. "The association between childhood obesity measures and adulthood knee pain, stiffness and physical dysfunction" (Poster presentation)

2014 Australian Rheumatology Association (ARA) Conference 2014, Hobart, Australia. "Patellar bone marrow lesions predict patellar cartilage defect progression, cartilage volume loss and knee pain in older adults" (Oral presentation)

2015 Australian Rheumatology Association (ARA) Conference 2015, Adelaide, Australia. "Disruptive pathology rather than degenerative or discrete tear are associated with knee pain, increasing bone marrow lesion volume and a proxy for total knee arthroplasty: longitudinal analysis from the osteoarthritis initiative" (Oral presentation)

Local

2012 Graduate Research Conference – Sharing Excellence in Research 2012, Hobart, Australia. "Childhood physical fitness predicts adulthood knee cartilage volume and bone area" (Poster presentation)

2013 Tasmanian Health Research Students Conference 2013, Hobart, Australia. "The association between childhood obesity measures and adulthood knee pain, stiffness and physical dysfunction" (Poster presentation)

2014 Tasmanian Health Research Students Conference 2014, Hobart, Australia. "Correlates of heel bone mass in young Adults: the role of cholesterol over 20 years from childhood to early adulthood" (Poster presentation)

Awards Resulting from the Thesis

2011 Tasmanian Graduate Research Scholarship, University of Tasmania, Australia

2012 Travel Award, Graduate Research Travel Fund, University of Tasmania, Australia

2013 Travel Award, European Union League Against Rheumatism (EULAR 2013)

2013 Emerging Researchers in Ageing (ERA) International Exchange Award, Centre of Excellence in Population Ageing Research, Australian Research Council

2014 State Finalist for Young Scientist Award, FameLab Australia International Competition

2014 Young Investigator Award from American Society for Bone and Mineral Research (ASBMR) to attend the European Calcified Tissue Society Ph.D Training Course and conference at Oxford.

2014 Best Clinical Paper Award 2013, Menzies Institute for Medical Research

2014 Educational Travel Award, Australian Rheumatology Association 2014.

2015 New Investigator Award, Australian Rheumatology Association 2015.

List of Abbreviations

2D	two-dimensional
3D	three-dimensional
ACL	anterior cruciate ligament
ACR	American College of Rheumatology
AKP	anterior knee pain
ARA	Australian Rheumatology Association
ASBMR	American Society for Bone and Mineral Research
ASHFS	Australian schools health and fitness survey
BMD	bone mineral density
BMI	body mass index
BML	bone marrow lesion
CATI	computer assisted telephone interview
CDAH	childhood determinants of adult health
CT	computed tomography
CRP	C-reactive protein
CI	confidence interval
CV	coefficient of variation
dGEMRIC	delayed gadolinium-enhanced magnetic resonance imaging of cartilage
DXA	dual-energy x-ray absorptiometry
FAI	free androgen index
GAG	glycosaminoglycan
GEE	generalised estimating equations
GIS	geographical information systems
GRE	gradient-recalled echo
HDL	high-density lipoprotein
ICC	intraclass correlation coefficient

IOTF	international obesity task force
IPAQ	international physical activity questionnaire
JSN	joint space narrowing
LBM	lean body mass
LDL	low-density lipoprotein
NHMRC	National Health and Medical Research Council of Australia
MOST	Multicenter Osteoarthritis Study
MRI	magnetic resonance imaging
OA	osteoarthritis
OARSI	Osteoarthritis Research Society International
OR	odds ratio
PCL	posterior cruciate ligament
PFPS	patellofemoral pain syndrome
PPMs	physical performance measures
PR	prevalence ratio
PRT	progressive resistance training
PWC_{170}	physical work capacity at a heart rate of 170 beats/min
RIA	radioimmunoassay
RCT	randomised controlled trial
RR	relative risk
SD	standard deviation
SES	socioeconomic status
SHBG	sex hormone binding globulin
USA	United States of America
WOMAC	Western Ontario and McMasters Universities Osteoarthritis Index
WORMS	Whole-Organ magnetic resonance imaging score

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Synopsis

Knee joint is commonly affected by osteoarthritis (OA) and the major risk factors for knee OA include age, female sex, obesity and injury. However, the role of these risk factors in childhood and how they affect the knee joint in adulthood have not been explored. The association of physical activity with knee OA is unclear with studies suggesting both beneficial and detrimental effects. This thesis examines the association of physical activity, physical fitness and obesity measures in both childhood and adulthood with knee joint structure and function in young adults. Specifically, each chapter of this thesis investigates how different factors such as physical performance and obesity measures in childhood relate to knee pain and knee cartilage volume measured using magnetic resonance imaging (MRI) in adults. In addition, the correlates of structural measures in young adults such as tibial cartilage volume and bone marrow lesions (BML) will also be explored in this young adult population-based sample. The summary of each chapter of the thesis is presented below.

Chapter 1 provides an overview of OA with a focus on the risk factors of knee OA. A working definition of knee joint health and the measures that reflect the knee joint health are provided. The economic impacts, burden of disease, symptoms, risk factors, and treatment and management options of knee OA are discussed. Lastly, this chapter presents an overview of childhood and early life risk factors that are directly or indirectly related to knee OA or knee OA markers in later life and provides a rationale for examining childhood risk factors with adulthood knee joint health.

Chapter 2 lists the research questions and hypotheses to be addressed in the thesis.

Chapter 3 describes the Childhood Determinants of Adult Health (CDAH) Knee Cartilage Study population and its design including the source study such as CDAH study and Australian Schools Health and Fitness Survey (ASHFS) of 1985. The protocols for measurement of predictor measures from all three studies and outcome measures from CDAH Knee Cartilage study which are the same in multiple chapters of this thesis are also discussed in this chapter. Additional factors which are unique to each chapter are described in more detail within the methodology section of the subsequent chapters.

Chapter 4 describes the relationship between childhood physical performance measures such as running time, sit-ups, leg muscle strength and physical work capacity at 170 beats per minute (PWC_{170}) collected in ASHFS of 1985 and tibial cartilage volume and tibial bone area measures in 328 CDAH Knee Cartilage study participants (mean age 36 years, range 31–41 years, 48% female) 25 years later. The participants underwent T1-weighted fat- suppressed MRI of their knees as part of CDAH Knee Cartilage study. Tibial bone area and cartilage volume were measured from MRI. Childhood measures were assessed in 1985 according to standard protocols. PWC_{170} and leg muscle strength were repeated after 20 years in their adulthood. In multivariable analysis, there were consistent positive associations of all childhood performance measures including PWC_{170} (beta [β]: 0.38 cm^2 per 10 watts, 95% confidence interval [95% CI]: 0.15, 0.60), leg muscle strength, long-run, short-run, and sit-ups with adult medial and total tibial bone area. Similarly, there were positive associations of PWC_{170} and sit-ups with adult medial tibial cartilage volume. The associations between childhood fitness measures and adulthood cartilage volume were independent of the adult attained fitness levels measured after 20 years. After further adjustment for tibial bone area, the association between PWC_{170} and medial and total (β: 0.08 cm^3 per 10 watts, 95% CI: 0.02, 0.10) tibial cartilage volume decreased in magnitude but remained significant. In conclusion, childhood physical performance measures, especially PWC_{170}, were associated with knee tibial bone area and cartilage volume in adulthood. The associations with cartilage volume appeared to be partially mediated by bone area. This suggests physical performance measures in childhood can independently influence adult knee structures.

Chapter 5 describes the associations between overweight measures in childhood and knee pain, stiffness and dysfunction among adults 25 years later. Participants broadly representative of the Australian population (n=449, aged 31-41 years, female 48%) were selected from the CDAH Knee Cartilage study. Height, weight and knee injury were recorded, and knee pain was assessed using the Western Ontario and McMaster University osteoarthritis index (WOMAC). Childhood height, weight and knee injury were measured according to standard protocols 25 years prior, and body mass index (BMI) and percentage overweight were calculated. The prevalence of knee pain was 34% and overweight in childhood and adulthood was 7% and 48%, respectively. Overall, there were no significant associations between childhood overweight measures and total WOMAC knee pain, stiffness and dysfunction scores. However, in males, overweight in childhood was associated with adulthood WOMAC pain (relative risk [RR]: 1.72, 95% CI: 1.11, 2.69),

and childhood weight and BMI were associated with WOMAC stiffness and dysfunction. Childhood weight, BMI and overweight were all associated with adulthood knee pain during walking in males and whole sample. Most of these associations were independent of adult overweight measures. Participants who were overweight in both childhood and adult life had a significant increase in the risk and prevalence of adulthood knee pain during walking (RR: 2.42, 95% CI: 1.06, 5.53). In conclusion, childhood overweight measures were significantly associated with adulthood knee mechanical joint pain, stiffness and dysfunction among males, independent of adult overweight, suggesting that childhood overweight may lead to later knee symptoms in men.

Chapter 6 describes the correlates of tibial cartilage volume such as body composition, hormonal and inflammatory factors measured 5 years prior. This chapter also explored if these factors contribute to the sex difference in tibial cartilage volume in young adults. Participants (n=328, aged 31-41 years, female 47.3%) were selected from the CDAH Knee Cartilage study. They underwent T1-weighted fat- suppressed MRI of their knees. Tibial cartilage volume and bone area were measured from MRI. Sex hormone binding globulin (SHBG) and testosterone in females only and C-reactive protein (CRP) and fibrinogen in both sexes were measured 5 years prior. Fat mass and lean mass were calculated from skin-folds. In multivariable analyses, correlates of tibial cartilage volume included lean body mass (β: 26.4 mm^3, 95% CI: 13.6, 39.1), fat mass (β: -11.8 mm^3, 95% CI: -22.2, -1.4), and fibrinogen (β: -146.4 mm^3, 95% CI: -276.4, -16.4) but not BMI, testosterone and CRP. In females, SHBG was positively associated with tibial cartilage volume (β: 0.67 mm^3, 95% CI: 0.14, 1.20) and free androgen index was negatively associated with lateral tibial cartilage volume (β: -0.04 mm^3, 95% CI: -0.07, 0.00). Males had 13% more tibial cartilage volume (500 mm^3) than females. The magnitude of this association decreased by 38%, 20% and 37% after adjustment for lean body mass, fat mass and fibrinogen, respectively. In conclusion, body composition, sex hormones and fibrinogen correlate with knee cartilage volume in young adult life. Sex difference in knee cartilage volume is contributed largely by variations in body composition and/or fibrinogen.

Chapter 7 describes the associations between physical activity and physical performance measured 5 years prior and tibial cartilage volume and bone area in young adults. Participants (n=328, aged 31-41 years, female 47.3%) were selected from the CDAH Knee Cartilage study. Tibial bone area and cartilage volume were measured from MRI. Physical activity (measured using long international physical activity questionnaire (IPAQ)) and performance measures (long jump, leg muscle strength, PWC_{170}) were measured 5 years

prior according to standard protocols. In multivariable analyses, total physical activity (min/week) (β: 0.30 mm^3, 95% CI: 0.13, 0.47), vigorous (β: 0.54 mm^3, 95% CI: 0.13, 0.94), moderate (β: 0.34 mm^3, 95% CI: 0.01, 0.67), walking (β: 0.40 mm^3, 95% CI: 0.07, 0.72) and IPAQ category (β: 182.9 mm^3, 95% CI: 51.8, 314.0) were positively associated with tibial cartilage volume but not tibial bone area. PWC_{170}, long jump and leg muscle strength were positively and significantly associated with both tibial cartilage volume and tibial bone area; and the associations with tibial cartilage volume decreased in magnitude but remained significant for PWC_{170} and long jump after further adjustment for tibial bone area. In conclusion, tibial bone area is affected only by physical performance and tibial cartilage volume is influenced by both physical activity and performance in younger adults. This suggests a beneficial effect of physical activity and physical performance on cartilage volume. Only physical performance measures were associated with tibial bone area suggesting other factors rather than physical activity may be important.

Chapter 8 describes the prevalence and correlates of BMLs in younger adults. Participants (n=328, aged 31-41 years, female 48.7%) were selected from the CDAH Knee Cartilage study. They underwent T1- and proton density weighted fat- suppressed MRI in their knees. BMLs, cartilage defects, meniscal lesions and cartilage volume were measured using these MR images. Knee pain was assessed by WOMAC scale and physical activity was measured by the short version of IPAQ at the time of MRI. Cholesterol levels were assessed 5 years prior. The prevalence of any BML was 17%. BML was positively and significantly associated with increasing age and previous knee injury (medial tibiofemoral only) but not with BMI. Moderate physical activity (prevalence ratio [PR]: 0.93, 95% CI: 0.87, 0.99) and high-density lipoprotein (HDL) cholesterol (PR: 0.36, 95% CI: 0.15, 0.87) were negatively associated with BML, while vigorous activity was positively associated with medial tibiofemoral BMLs. BMLs were associated with more severe total WOMAC knee pain (>5 vs ≤5, PR: 1.05, 95% CI: 1.02, 1.09) and WOMAC dysfunction (PR: 1.75, 95% CI: 1.07, 2.89), total knee cartilage defects (PR: 2.65, 95% CI: 1.47, 4.80) and total meniscal lesion score (PR: 1.92, 95% CI: 1.13, 3.28). In conclusion, BMLs in young adults are associated with knee symptoms and other knee structural lesions. Moderate physical activity and higher HDL cholesterol are beneficially associated with BMLs, in contrast, vigorous physical activity is weakly but positively associated with medial tibiofemoral BMLs, suggesting that BMLs are modifiable in young adults.

Chapter 9 summarises the findings of the thesis and also provides a number of potential directions for future research based on these conclusions.

Chapter 1 - Introduction

1.1 Knee joint

The knee joint is one of the largest and complex joints in the human body and is an important joint for locomotion [1]. Knee joint consists of the junction of femur, tibia and patella bones [2] (Figure 1.1a). Therefore, knee joint can be mainly categorised into medial tibiofemoral, lateral tibiofemoral and patellofemoral compartments [1]. Knee joint is connected to the tendon of the leg muscles, which makes the joint movement possible. Anterior cruciate ligament (ACL), posterior cruciate ligament (PCL) and collateral ligaments stabilise the femur and tibia within the knee joint. Two C-shaped menisci act as shock-absorbing cartilage in transmitting the load effectively from femur through tibia [3](Figure 1.1b). The smooth articular cartilage surface on the tibia, femur and under surface of patella along with the synovial fluid allow the smooth gliding between these bones in the knee joint. The fat pads in the knee, especially infrapatellar fat pad, act as shock-absorbing cushion in the joint and effectively distribute the synovial fluid within the joint [4].

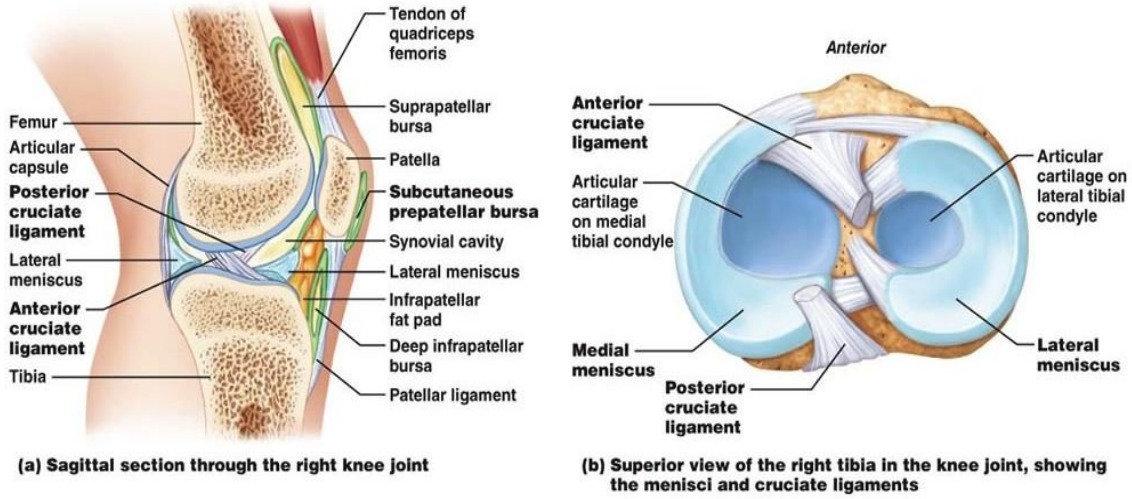


Figure 1.1 Anatomy of a healthy knee joint

(Reproduced with permission from Greg Linstead, Science Aspects 2 © Pearson Australia)

1.1.1 Knee joint health markers

Knee joint health is mainly indicated by the structural and functional integrity of its components. Any pathology in the structure of components of knee joint can result in the functional incapability of the joint resulting in joint disability. Therefore, any objective abnormality in the morphology and patient-reported symptoms (knee pain, stiffness and dysfunction) can be a marker of knee joint health. Most of these abnormalities are associated with the development and progression of major joint diseases including osteoarthritis (OA) [5], which can be clinically developed in later life.

Advanced medical imaging techniques have enabled us to have a closer look at different knee structures non-invasively. X-ray is a basic diagnostic imaging tool and can visualise bony structures and fractures but is limited as it is unable to visualise soft tissues. People who present with acute knee injuries are most likely to have soft tissue rather than osseous injuries, and fracture is present often accompanying with soft tissue injury. Magnetic resonance imaging (MRI) enables the clear visualisation of the soft tissues including cartilage and is a useful tool for the detection of early knee OA [6]. Computed tomography (CT) has a lesser role in the assessment of knee joint structures, though it is useful in demonstrating subtle bony injury and loose bodies within the knee joint.

1.1.1.1 Cartilage health markers

Cartilage can be visualised clearly using MRI. Advanced imaging software can process these images to quantify the amount of cartilage in the knee to yield cartilage volume of the tibia, femur and patella. A higher cartilage volume in a knee where there is no abnormality of cartilage may predominantly reflect healthier hyaline cartilage. There is evidence to suggest that increased cartilage volume is associated with reduced radiographic OA [7, 8], cartilage defects [8] and knee symptoms [9]. Similar as the peak bone mass that is a strong predictor of future risk of osteoporosis in older people [10], a higher cartilage volume should be protective against the development of OA in later life. Therefore, identifying the factors that are associated with “peak” cartilage volume in young adults can identify the mechanisms related to the development of knee OA.

Cartilage defects are one of the early signs of abnormalities to cartilage and are characterised by surface fraying and signal intensity changes in the MR image of cartilage. Cartilage defects can predict other knee OA features including cartilage volume loss [11] and can be an independent risk factor for the development of OA. The level of evidence for the association between cartilage defects and pain is conflicting, and 50% of studies show

a positive association in a systematic review [12]. However, higher cartilage defect scores (8-15) were associated with a six-fold increased risk of joint replacement over 4 years compared with those with lower cartilage defect scores (2-7) in knee OA patients [13].

1.1.1.2 Subchondral bone health markers

Cartilage bears only 1 to 3 percent of the body load contrary to periarticular bone which bears 30-50 percent of the load. Recent researches have identified the importance of bone in the pathogenesis of OA. Osteophytes are bony protrusion from the margins of the bone and are the hallmark features of knee OA in radiographs. Theoretically, large osteophyte increases the stability of the joint, especially in destabilised joint resulting from injury or disease like OA as it increases the joint surface area for a more stable joint [14]. Only a few studies have found an independent association of osteophytes with knee pain or the progression of OA [12, 14]. However, recent evidence suggests that osteophytes may be involved in the incidence of knee OA [15].

The surface area of the joint can be measured using MRI, and evidence has suggested that the increased tibial bone area is associated with knee OA in older populations [16, 17]. Changes in bone area discriminated people with OA from controls and was greater in patients with OA progression [18]. Tibial bone area is positively associated with cartilage defects and cartilage volume loss in older adults [17] and has been shown to be an independent predictor of knee replacement over 4 years [19]. Tibial bone area increase in older populations appears maladaptive and is possibly due to the disproportionate load transmission on the tibial bone [20]. However, the change in subchondral bone size in OA warrants further investigation. There is, as yet, no evidence to suggest whether increased tibial bone area among young adults is associated with knee OA in later life. Exercise during childhood and adolescence is associated with increased cortical bone size which persists over years leading to increased bone mass [21, 22]; therefore, increased bone area during the growth may reflect an adaptive change. In younger life without OA, a greater tibial plateau area will keep stresses in the joints within limits as higher loads are distributed over a greater surface area. Hypothetically, a greater tibial plateau will limit stresses in the joints because higher loads are distributed over a greater surface [23]. Similarly, bone size in young adults can be the result of genetics and adaptations to physical stimuli during skeletal growth [22], and increased tibial plateau area in younger adults may be protective by reducing stresses in the joints [20]. A small cross-sectional study of adult triathletes found no difference in cartilage volume or

thickness, but the bone size was larger among triathletes than inactive volunteers [20]. Therefore, a higher knee bone area in young adults could be protective and may contribute to the development of larger healthy cartilage.

Subchondral bone marrow lesions (BMLs) are represented as increased signal intensity area adjacent to periarticular bone in T2/proton density-weighted MRI. Increasing evidence suggests a causal role of BMLs in the pathogenesis of OA. Studies have suggested that BMLs are associated with cartilage defect progression and cartilage volume loss [24] and can predict knee joint replacement [25]. BMLs can progress and regress over time which makes BML an important imaging biomarker for OA [26, 27]. Reducing BML size may be an important therapeutic goal for the future disease-modifying drug invention. Therefore, identifications of factors associated with BMLs are of importance in OA research.

Subchondral bone is richly innervated with nociceptive pain fibers [28]. Thus, subchondral bone could be a source of knee pain. There is increasing evidence to suggest that BMLs are associated with knee pain [29-32] in older adults and knee OA patients, though some studies did not establish this association [33, 34]. A systematic review suggested that there was a moderate evidence for the association between BMLs and knee pain [12]. BML regression is associated with a decrease in knee pain [35, 36]. This also makes BMLs an attractive target for the treatment of knee OA [37] and being used in clinical trials as an outcome measure [26, 27].

There are only very few studies that explored the correlates of BMLs in a sample that includes young adults. A study conducted within the Melbourne Collaborative Cohort Study (aged 40-69 years) on subjects with no clinical knee OA reported that the risk factors for BMLs included age, male gender, and body height [38]. Another study that aimed at the natural history of BMLs in the same cohort found that the incident BMLs were associated with increased body mass index (BMI) and the development of knee pain [37]. Similarly, Guymer et al conducted a study in healthy middle-aged women (40-67 years) and found that BMLs were associated with risk factors (height and weight) for knee OA, and the early structural changes of knee OA [39]. Large BMLs were associated with both progression of cartilage defects and rapid cartilage volume loss in the same cohort when they were followed over 2 years [40]. However, these studies were conducted on healthy subjects aged from 40 to 69 years. A similar study was conducted in the offspring of the patients who underwent total knee replacement for knee OA and matched controls (aged 26-61 years), and reported that the knee injury was associated with BMLs [41]. Foong et al described the natural history of BMLs over 8 years from the same cohort and

reported that the change in BMLs were predicted by BMI and strenuous activity [42]. An increase in BML size or new BML resulted in an increase in pain especially in males and those with a family history of knee OA [42]. However, this study included a wide range of participants aged from 26 to 61 years. No studies have explored the correlates of BML in a young population-based sample.

1.1.1.3 Other knee health markers

Other knee joint health abnormalities such as meniscal lesions, synovitis (joint effusion), ligament abnormalities (including cruciate and collateral) and infrapatellar fat pad abnormalities (size and intensity changes) are also visualised in MRI. These abnormalities in the knee are also associated with the development and progression of knee OA. Therefore, these abnormalities can also be considered as markers of knee OA in subjects who do not have an established knee OA.

1.2 Osteoarthritis

Osteoarthritis (OA) is characterised by gradual deterioration and changes in the structures and functions of the articular joint causing pain and disability. OA is considered as a disease of the whole joint including articular cartilage, subchondral bone and meniscus leading, eventually, to total joint replacement in some cases. There is an increasing evidence to suggest that ligaments, fat pads, synovium and, periarticular muscles are also involved in the pathogenesis of OA (Figure 1.2).

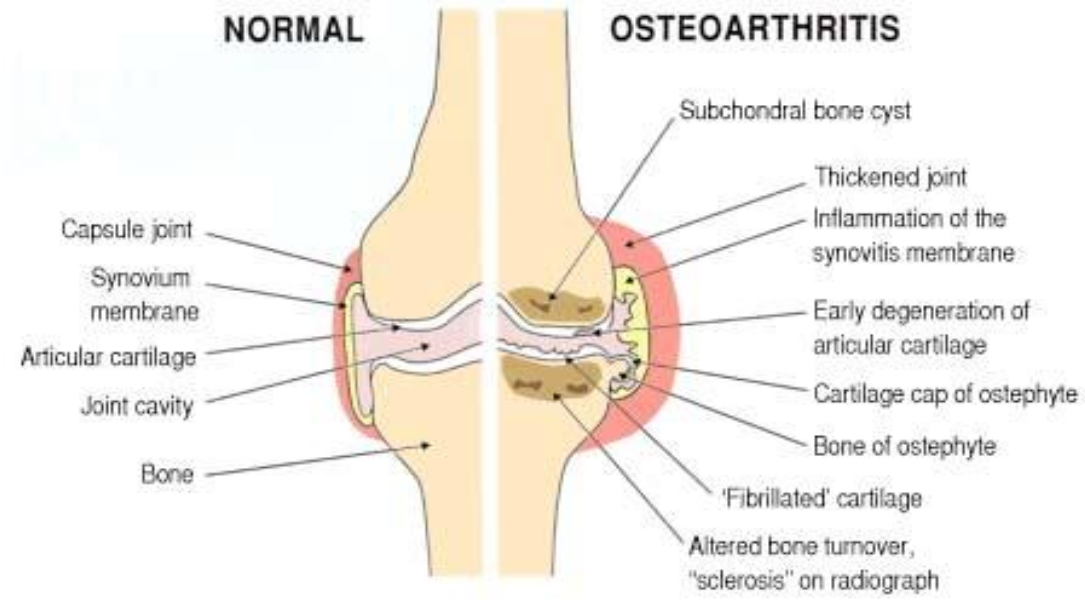


Figure 1.2. Schematic drawing of the knee joint showing normal and osteoarthritis changes to all structures in the knee

(Figure with no copyright and taken from http://www.mendmeshop.com/knee)

OA is the most common joint disorder in the world. It is one of the most frequent causes of pain, loss of function and disability among adults in Western population [43]. The global prevalence of radiographically-confirmed symptomatic knee and hip OA in 2010 was estimated to be 3.8% and 0.85%, respectively. It was higher in females (mean 4.8%) than in males (mean 2.8%) [44]. OA ranked 13th in the top 25 causes of global years lived with disability and the 4th leading cause that showed an increase in the years lived with disability share form 1990 to 2013 [45].

In Australia, 14.8% of people (or around 3.3 million people) had arthritis, with more than 55.9% affected by OA (1.9 million people) costing the health system $3.75 billion and the economy around $23.9 billion annually [46]. This cost is expected to rise due to increasing number of joint replacements. Over 85,000 knee and hip replacement procedures (majority due to OA) were performed in Australian hospitals during 2012, each costing an average of $15,000–$31,900 [47]. The number of joint replacements being performed is increasing at a rate of 10% per annum and by 2018, it is expected that the number of joint replacements will be double the number performed in 2012 [48].

OA is the leading cause for chronic pain in general practice patients in Australia [49] and accounts for 63% of hospital in-patient expenditure. Conventional treatment accounts for 75% of the aged care expenditure; considerably, above its prevalence share of

42% indicating the greater impact it has on older Australians [50]. 30% of hospital outpatient expenditure is attributable to OA [50].

1.3 Knee OA

OA can affect any joint but occurs most often in the knees, hips, finger joints and big toes. Knee OA is the most common form of OA, and nearly one in two older adults are affected by symptomatic knee OA by the age of 85 [51]. Approximately 25% of people 55 years of age or older have had knee pain on most days in a month in the past year, and about half of them have radiographic knee OA [43]. Currently, there are no registered disease-modifying knee OA drugs. Therefore, there is an urgent need for research that investigates innovative and cost-effective approaches to prevent or slow down the progression of knee OA.

1.3.1 Major symptoms

Pain is one of the most common symptoms of knee OA. Knee pain is associated with considerable reduction in functional ability, which in turn strongly predicts future disability and dependency [52]. During a one-year period, 25% of people over 55 years demonstrated persistent episode of knee pain, in whom about one in six had to consult their general practitioner about it in the same time period [53]. The prevalence of symptomatic knee OA and knee pain has increased substantially over the past 20 years, independent of age and obesity [54]. After adjustment for both age and BMI, the prevalence of knee pain increased by about 65% from 1974 to 1994 among Non-Hispanic White and Mexican men and women and among African American women [54]. Similarly, the prevalence of knee pain and symptomatic knee OA approximately doubled in women and tripled in men over a 20- year period in Framingham Osteoarthritis Study from 1983 to 2005 [54]. However, after age adjustment, additionally adjusting for BMI resulted in a 10–25% decrease in the prevalence ratios for knee pain and symptomatic knee OA [54]. Knee pain is a common cause for seeking medical advice in general population [55]. Patients with knee OA also experience joint stiffness, tenderness, swelling, crepitus, instability, and muscle weakness. These symptoms leads to limitations of functions of the joint mainly affecting the movement leading to physical and psychological disability, and impaired quality of life. Because of its prevalence and the frequent disability that accompanies disease in the knee and hip, OA accounts for more trouble with climbing stairs and walking than any other disease [56].

1.3.2 Risk factors

OA was regarded as a disease arising from "wear and tear" of the joint. This paradigm was mainly based on the observation that chondrocytes, the only cell type present in cartilage, have very low metabolism activity with no ability to repair cartilage [57]. Moreover, unlike all other tissues, articular cartilage, once damaged, cannot respond by a usual inflammatory response because it is non-vascularised and non-innervated [57]. There were arguments regarding the name 'osteoarthritis' as it was suggested that no inflammation would be involved to indicate 'itis'. The term 'osteoarthrosis' was in use to suggest the cartilage degradation without inflammation. However, recent studies suggest that metabolic and low-grade inflammatory processes are involved in OA. All these suggest that OA is a complex disease with multifactorial aetiology and involves biomechanical, metabolic and inflammatory processes.

Age remains one of the strongest risk factors for the development of OA [56]. The ageing changes observed in the cells and extracellular matrix of joint tissues likely increase the susceptibility of older adults to OA when other OA risk factors are also present [58]. There seems to be a 'non-linear' relationship between age and knee OA incidence with a sharp increase in incidence between the ages of 50 and 75, but limited increase above the age of 75 [59].

Prevalence of OA in most joints is higher in men than in women before 50 years of age. However, after 50 years, women are more often affected with the hand, foot, and knee OA than men [60] and also have more severe OA [61]. The high incidence of OA in women just after menopause suggests that hormone mechanisms (estrogen deficiency) may play a role in causing disease. Cicuttini et al reported that the serum free testosterone level was positively associated with tibial cartilage volume in a cross-sectional study in healthy men; [62] however, serum free testosterone was associated with an increased rate of cartilage loss in its follow-up study [63]. A similar study reported a positive association between sex hormone binding globulin (SHBG) and patella bone volume, although they found no associations with the tibial and patellar cartilage volume [64]. However, a systematic review found conflicting evidence and suggested that there was no clear association between female hormonal factors and OA of the hand, hip and knee [65].

Other systemic risk factors include genetics / inheritance. The contribution of heritability and possible genetics to the occurrence of OA varies by joint, with a higher percentage of the hand and hip OA due to inheritance than knee OA [66]. Some development conditions such as malalignment has been proposed as a risk factor for the

progression of the knee OA, but the evidence is not strong enough to support a causative role in the incidence of knee OA [67].

Obesity or overweight is the strongest and well-agreed risk factors for the development and progression of OA, especially for knee OA. A recent systematic review has suggested consistent evidence for the association between obesity (pooled odds ratio (OR) of 2.66) and overweight or obesity (pooled OR of 2.10) and knee OA [59]. There was a dose response relationship between BMI (continuous scale) and development of knee OA: for every 5 kg/m^2 increase in BMI, the relative risk of developing knee OA increased by 37% (OR 1.4) [68]. Using MRI, longitudinal studies have shown that BMI is associated with an increase in knee cartilage defects in healthy subjects [69]; however, the association of BMI with knee cartilage volume is controversial. Most of the studies failed to demonstrate an association of BMI with cartilage volume in osteoarthritic [70] or healthy knees [71, 72] although a few found that BMI was negatively associated with cartilage volume [62]. We reported that BMI was associated with knee cartilage loss only in people within the highest tertile of baseline knee cartilage volume [73]. These inconsistencies may be due to the inability of BMI to differentiate lean mass from fat mass. There is evidence to show that muscle mass [74] and muscle strength [75] are associated with more cartilage volume.

Joint injury is associated with an increased risk of knee and hip OA. Knee injury may result in transarticular fracture, meniscal tear requiring menisectomy or ligament injury requiring repair that can result in increased risk of knee OA development and musculoskeletal symptoms [76]. The extent of heterogeneity in the studies that explored the association between injury and knee OA is high and the pooled OR ranges from 2.83-4.2 [59, 77]. Occupational activity that involves physically demanding activities (repetitive knee bending and kneeling) was also found to be a risk factor for knee OA [59, 78].

Life style risk factors have major roles in OA incidence and progression. Diet also has a role to play, as deficiencies in Vitamin D [79], Vitamin K [80], vitamin C [81], vitamin E [82] are associated with knee pain and progression of OA. In fact, dietary fatty acids, minerals and carbohydrates may have an independent role in the pathogenesis of OA apart from their influence on obesity [83]. Serum cholesterol is considered as a risk factor for OA and statin use has been found to be protective on the incidence and progression of OA [84].

Other life style factors such as physical activity, exercise, alcohol and smoking are also proposed as risk factors in knee OA. Racunica et al reported that tibial cartilage volume increased with frequency and duration of vigorous activity reported 10 years

previously, as well as recent vigorous activity in the 7 days prior to MRI in healthy, community-based adults with no history of knee injury or disease [85]. A systematic review considering the different markers of knee OA including MRI markers have found that physical activity may increase the risk of osteophytes, but reduce the risk of cartilage defects [86]. However, the evidence for effects of physical activity or exercise on knee OA remains inconclusive, including levels of physical activity and sport specificity in individuals who do not suffer an injury [77, 87].

While physical activity reflects a behavior, physical performance is operationalized as several measurable health-related phenotypes including cardiorespiratory fitness and muscle performance which have both genetic and environmental components. Physical performance measures could be a proxy measure of physical activity but there is only modest correlation between physical activity and physical fitness [88]. Numerous cross-sectional studies have established that quadriceps muscle weakness is present in individuals who have knee OA even in the early stages of cartilage thinning [89, 90]. Longitudinal studies in older and middle-aged adults have demonstrated a protective effect of performance measures including muscle strength and cardiorespiratory fitness on knee structures [75, 91, 92]. In elderly women, knee extensor strength was 18% lower at baseline among subjects who developed incident knee OA than among the controls [93]. However, this finding did not reach statistical significance and there was no association in men. In addition, analyses of strength did not adjust for contralateral OA or for other potential confounding factors. In another study that followed participants for a mean duration of 14.4 years, the greater quadriceps muscle strength at baseline was associated with a 55% to 64% reduced risk of self-reported hip or knee OA in women [94]. In the Multicenter Osteoarthritis Study (MOST), neither knee extensor strength nor the hamstring: quadriceps ratio was predictive of incident radiographic tibiofemoral OA. However, compared with the lowest tertile, the highest tertile of knee extensor strength protected against development of incident symptomatic whole knee OA in both sexes [95]. In the same cohort, women in the lowest tertile of quadriceps strength had an increased risk of whole knee joint space narrowing (JSN) (OR = 1.66) and tibiofemoral JSN (OR = 1.69) but this was not found in men [96]. Similarly, studies using MRI reported that the increased muscle mass was positively associated with medial tibial cartilage volume and a loss of muscle mass over 2 years was associated with an increased loss of medial and lateral tibial cartilage volume over 2 years, after adjusting for confounders [97]. However, the quadriceps muscle strength did not predict tibiofemoral cartilage loss 2.5 years later in older adults with radiographic knee OA and frequent symptoms at baseline but predicted

less patellofemoral cartilage loss [92]. In middle-aged adults predominantly without radiographic knee OA at baseline, greater lower limb strength was protective against medial and lateral femoral cartilage volume loss in both men and women, and that muscle weakness accounted for the higher rate of cartilage loss in women [98]. In the same cohort, lower-limb muscle strength at baseline was positively associated with percent-per-year changes in total tibial cartilage volume (r = 0.13) and physical fitness especially physical work capacity at 170 heart beat per minute (PWC_{170}) was associated with a reduced tibial cartilage volume loss over 2 years [75]. However, a randomised controlled trial (RCT) evaluated strength training programme over 30-month in older adults and found that strength training had no effect in those with radiographic OA, but significantly increased the rate of JSN in those with normal radiographs at baseline [99].

Smoking seems to have a protective effect on the knee joint including reduced risk of knee replacement surgery [100-102]. However, MRI findings suggest a detrimental effect of smoking on cartilage including cartilage defects[103] and cartilage volume loss [104]. Even if smoking offers some benefit in reducing the risk of developing OA, it is hardly likely to be considered a valid population-based preventative strategy, given the associated overall health damage that develops due to smoking. Additional risk factors include low socioeconomic position and other co-morbidities such as cardiovascular disease (hypertension and coronary heart diseases).

1.4 Influence of early life on knee OA

Knee OA is a disease mainly affecting the old people. Considering the mechanical aspect (imbalance in the movement and physical force transmission through the joint) of this disease, childhood risk factors such as obesity would be of great importance in the development of this disease. However, it is difficult to identify the risk factors that have a life course influence on the development of OA, mainly due to the requirement of a long-term follow-up from childhood to adulthood. Therefore, only very few studies have reported the childhood risk factors for OA. Part of the reasons for this is due to the absence of a consented marker for joint health or early markers of OA, which can predict the development of OA in later life. Understanding of the early joint changes associated with OA are incomplete and research into the development and validation of biomarkers including imaging biomarkers, and other methods of detecting OA in its early stage is ongoing. There are plenty of studies exploring the childhood risk factors including birth weight in osteoporosis, and is mainly due to the identification of peak bone mass as a

predictor for future osteoporosis or fracture risk [10]. Such markers of joint health are not yet established in OA research; therefore very few studies have looked at the effects of childhood or early adulthood risk factors on development or progression of OA in later life.

An alternative approach is to incorporate advanced imaging techniques to already existing birth cohorts, which have reached their adulthood. These imaging techniques such as MRI can identify early markers of joint abnormality that can predict the development of OA in later life. Cartilage volume is being proposed as a marker of knee joint health, similar as the peak bone mass that is a strong predictor of future risk of osteoporosis in older people [10], which can protect against the development of knee OA in later life. This thesis is based on implementation of MRI in an already existing childhood cohort study, i.e., the Childhood Detriments of Adult Health (CDAH) study, with a focus on identifying the early life risk factors for knee OA.

1.4.1 Early life risk factors of knee OA

Identifying modifiable risk factors for knee OA early in life itself is of high priority as these risk factors are potentially reversible and can even prevent or delay this major, but poorly understood, public health problem. The current evidence (direct and indirect) for early life risk factors (childhood, adolescence and early adulthood) that are related to the development and progression OA in later life are briefly discussed here.

1.4.1.1 Early life obesity and knee OA

Obesity is a major public health problem among adults and, more recently, among children around the world. Obesity has also been strongly associated with many disease conditions and is also associated with knee OA than any other OA [78]. Obesity is a well-known risk factor for incident knee OA in adults, but the evidence of childhood obesity leading to later OA is largely unexplored.

The major importance of childhood overweight arises from the fact that it predicts adult obesity [105]. Adolescents who were overweight were almost 18 times more likely than their leaner peers to be obese in early adulthood [106]. Obesity in childhood was strongly predictive of obesity in early adulthood [107]. This tracking of obesity makes it difficult to identify the independent influence of childhood obesity on health conditions including OA.

Wills et al suggested that obesity from childhood might have an accumulative effect on knee OA development (Figure 1.3). BMI in men as early as 20 years old and women as early as 15 years old was associated with increased knee OA at the age of 53 years (Figure 1.4) [78, 108]. Changes in BMI from childhood in women and from adolescence in men were also positively associated with knee OA (Figure 1.5). Prolonged exposure to high BMI throughout adulthood carried the highest risk and there was no additional risk conferred from adolescence once adult BMI had been accounted for [108]. However, they used symptomatic criteria to define knee OA at 53 years of age and did not have the radiographs to assess or confirm the structural pathology. They defined prevalent symptomatic knee OA at one time point (53 year) and therefore could not comment on the influence of childhood or adolescent BMI on knee health of young adults. The same study group previously reported that hand OA in men at the age of 53 years was associated with increased weight at the age of 26, 43, and 53 and with decreased weight at birth in men. The highest risk for hand OA was observed in men who had been heaviest at age 53 years and lightest at birth [109].

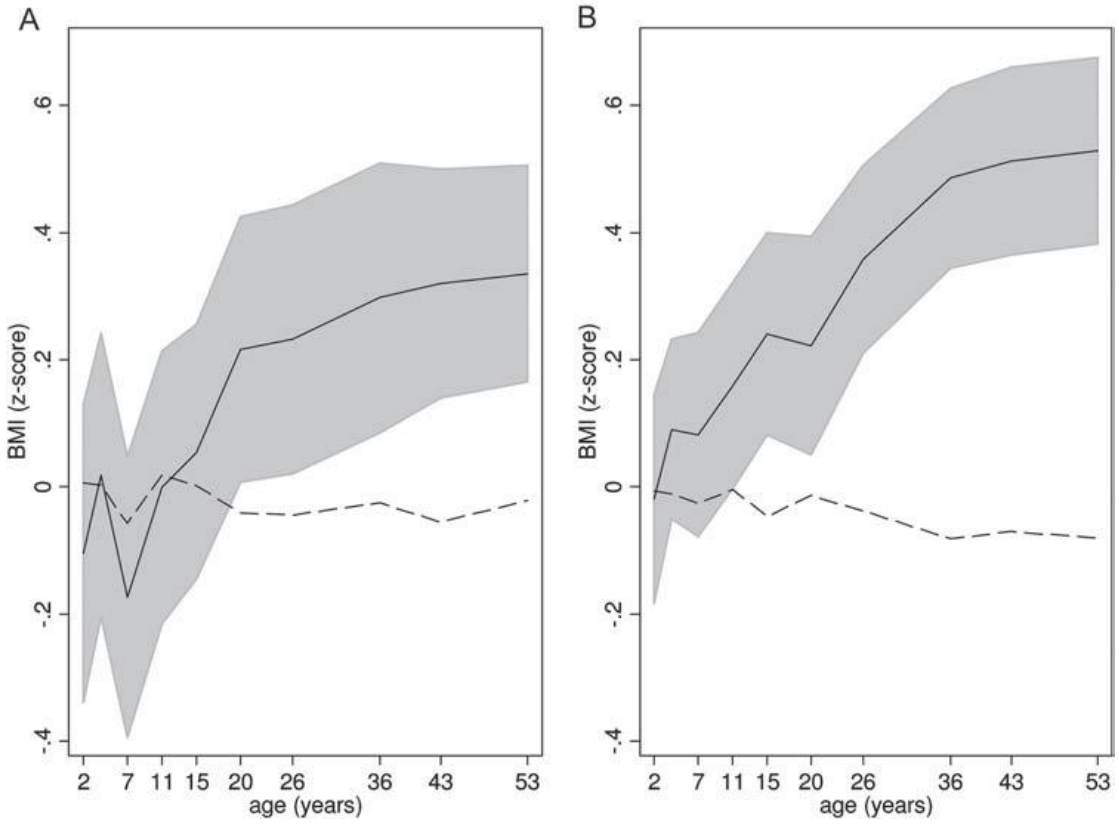


Figure 1.3. Mean lifetime BMI z-score among those with knee osteoarthritis

Mean lifetime body mass index (BMI) z-score and 95% CI (shaded area) in men (A) and women (B) among those with knee osteoarthritis (OA; solid line) at age 53 years. The dashed line is the mean BMI pattern in individuals without knee OA at age 53 years. (Reproduced from Wills et al [108] with permission from BMJ Publishing Group Ltd.)

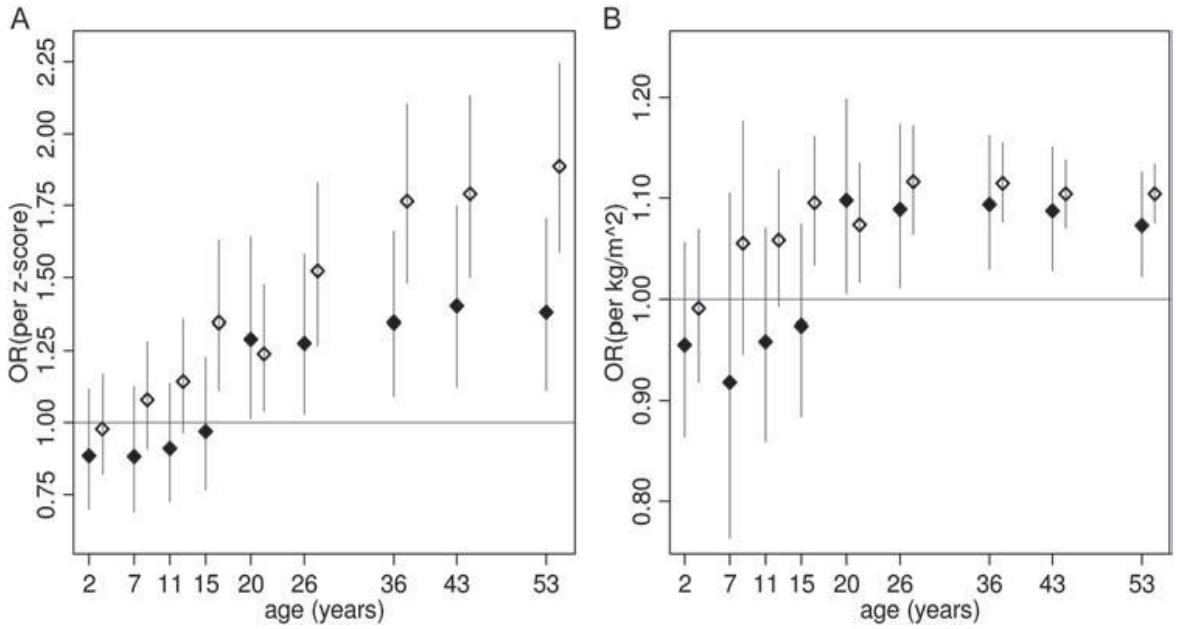


Figure 1.4. Odds ratios for knee osteoarthritis per z-score increase in BMI at each age and per kg/m^2 increase in BMI

Odds ratios (OR) for knee osteoarthritis in men (filled markers) and women (open markers) per z-score increase in body mass index (BMI) at each age (A) and per kg/m^2 increase in BMI (B). Adjusted for activity levels and occupation (manual/non-manual). Evidence for a sex interaction using BMI z-scores at ages 15 years (p=0.035) and 53 years (p=0.025) and weak evidence at ages 36 years (p=0.051) and 43 years (p=0.089). Evidence for sex interaction using non-standardised BMI at age 15 years (p=0.043). (Reproduced from Wills et al [108] with permission from BMJ Publishing Group Ltd.)

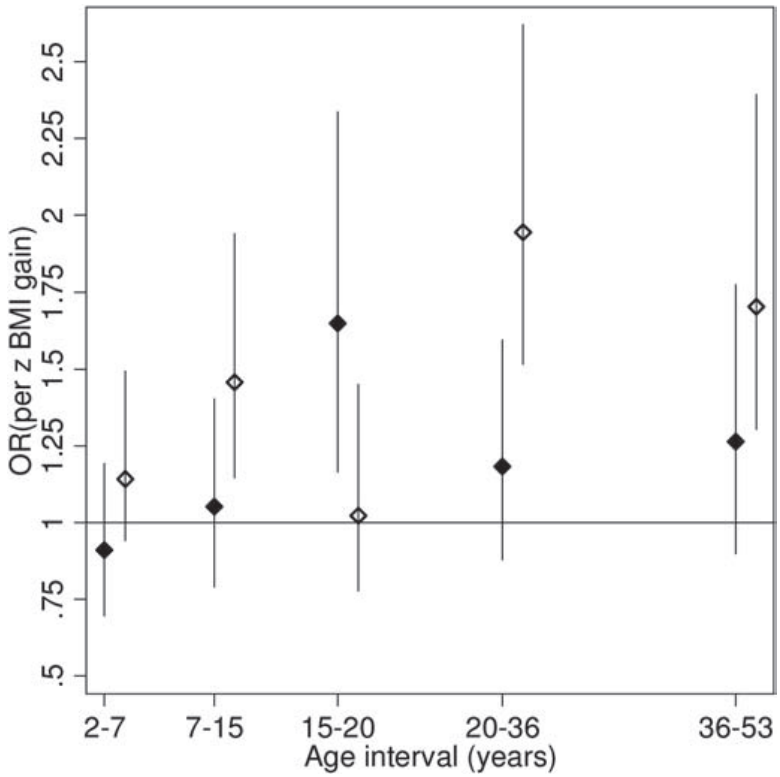


Figure 1.5. Association between conditional BMI change and knee osteoarthritis from childhood to mid-adulthood

Association (OR) between conditional body mass index (BMI) change (per z-score increase) and knee osteoarthritis in infancy (2–7 years), childhood/adolescence (7–15 years), adolescence to young adulthood (15–20 years), early adulthood (20–36 years) and mid-adulthood (36–53 years) in men (filled markers) and women (open markers). Each period of BMI change is adjusted for BMI at the beginning of the interval. Adjusted for activity levels and occupation (manual/non-manual). Sex interactions: 7–15 years: p=0.088; 15–20 years: p=0.035; 20–36 years: p=0.012. (Reproduced from Wills et al [108] with permission from BMJ Publishing Group Ltd.)

These results support the fact that systemic as well as mechanical factors underlie the link between OA and body weight and suggest that developmental influences may be important [109]. For the weight-bearing joints, the combination of increased load and changed joint biomechanics can be regarded as an underlying mechanism for the association between obesity and OA. Obese individuals showed altered biomechanics during everyday movements and these altered biomechanics could initiate OA by changing the load-bearing regions of the articular cartilage in the weight-bearing joints [110]. Systemic factors such as adipokines and other inflammatory markers associated with obesity can also influence the association of obesity with OA as suggested by a higher prevalence of non-weight bearing hand OA in obese patients.

Childhood obesity leads to childhood pathologies that might indirectly relate to knee OA. There is evidence for a higher prevalence of lower extremity malalignment, fractures and musculoskeletal pain among obese children than among normal weight children [111, 112]. Studies have suggested that the association of obesity with knee OA progression is largely mediated by knee alignment [113, 114]. Therefore, obesity related malalignment is important in the context of a life-course approach studies for knee OA. A recent study exploring the knee alignment among obese and non-obese children found that the mean alignment was similar between obese and non-obese subjects [115]. However, in stratified analysis, there was a significantly greater variability in knee alignment among females at higher BMI Z-score and greater valgus alignment in obese adolescents in late puberty [115]. A similar study using dual-energy X-ray absorptiometry (DXA) detected a greater prevalence of lower extremity malalignment, mostly valgus deformity, in overweight children [111]. The major limitation of these studies is the use of DXA for assessment of alignment, which needs validation against longstanding radiographs. It is possible that a mild malalignment with excess weight loaded across the joints over time may be sufficient to contribute to the increased prevalence of knee pain and later knee OA. However, further longitudinal studies are required to determine whether childhood obesity is a risk factor for progressive malalignment that predisposes to pain and risk of early OA.

Injury is one of the causal risk factors of OA. Early life injury, although less harmful, is still associated with knee OA. There is evidence that greater BMI in childhood is associated with increased odds of lower extremity injuries and pain [116]. Similarly, increasing BMI is associated with increased odds of lower extremity fractures including foot, ankle, leg, and knee among children [117]. These injuries and fractures resulting from higher obesity may lead to knee OA in later life.

Prevalence of OA is higher among patients with cardiovascular diseases, and they may share some common mechanisms. Excessive weight significantly increases the risks for cardiovascular diseases in school-aged children [118]. It is highly possible that excess weight during childhood has an indirect impact on OA in later life through the shared mechanisms between OA and cardiovascular diseases.

1.4.1.2 Early life obesity and knee pain

Up to half of people aged 50 years or over reported having knee pain during the last one year, and a quarter reported having severe and disabling knee pain [119]. Knee pain is the commonest presenting feature of OA, although knee pain is only weakly related to the structural damage seen in OA [120]. In the context of OA, knee pain results from a complex interplay between structural damage, peripheral and central pain processing, culture, gender, and psychosocial factors [121]. Age, previous knee injuries, overweight, and knee-straining work were found to be the risk factors, which contributed to the incidence of knee pain and these risk factors for self-reported knee pain seemed to be highly similar to the risk factors for knee OA [122]. Therefore, knee pain should be considered an important marker along with other knee OA features.

The knee joint is commonly affected by pain in both overweight paediatric [111] and adult populations [123]. Studies in adults have established a link between obesity and the subsequent development or progression of knee pain [119, 124]. A recent systematic review that studied the relationship between overweight and various musculoskeletal complaints in children suggested that there was moderate evidence for a positive association between overweight during childhood and musculoskeletal pain with a relative risk of 1.26 [112]. However, there was no relationship between body composition or body mass and patellofemoral pain in young school basketball players [125] or knee pain among school children [126].

The effect of childhood obesity on the knee joint can persist into adulthood and can lead to higher knee pain in later life. Following up birth cohorts with OA measures is a way for identifying the life course risk of obesity on knee joint. A study from 1956 British Birth Cohort by McFarlane et al explored the association of weight from childhood to adulthood (BMI at 7, 11, 16, 23, 33 and 45 years) with adulthood knee pain at 45 years. They found a higher risk of adulthood knee pain for the obese category relative to the underweight in each age group [127]. Childhood BMI was associated with adult knee pain, but this association was dependent on adulthood BMI [127]. However, BMI in the early

20s was an independent predictor of knee pain at 45 years [127]. This study had some limitations because adult BMI categories were used for the definition of overweight and obesity in childhood, the reference category for comparison was an underweight group rather than normal, men and women were not separated for analyses, and they did not use a validated and detailed scale for knee pain assessment such as a Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scale.

Knee pain is not an uncommon feature in early life [128]. Studies have found that the prevalence of knee pain among children are around 3.9% and in adolescents around 18.5% [126]. There are studies that reported even higher pathological knee pain in adolescents as high as 28.5%-31% [129, 130]. Knee pain in early life can result from many conditions that are not related to OA in later life. The most common causes of knee pain in early life include patellar subluxation, Osgood-Schlatter lesion (localised pain at the tibial tuberosity where the quadriceps muscles insert and is mostly associated with a growth spurt), patellar tendonitis, arthritis (bacterial, viral or inflammatory), referred pain from the hip, osteochondritis dissecans (bone and cartilage in the joint becomes calcified and may produce loose fragments of bone that can become caught), tumours and malignancies. Similarly, anterior knee pain (AKP) is a broad symptom classification and it does not imply any particular diagnosis or physical condition and is likely to be multifactorial [131]. Patellofemoral pain syndrome (PFPS) accounts for almost 50% of the nonspecific knee pain in adolescents [132]. Major symptoms of PFPS are diffuse peripatellar and retropatellar localised pain, pain provoked by ascending stairs, descending stairs, squatting, cycling and sitting with flexed knees for prolonged periods of time [133]. Similar to OA in older adults, prevalence of PFPS has been found to be higher in females with prevalence approximately 1.5-3 times higher than males in athletic populations [134]. It is possible that these knee pain symptoms persist into later life and may lead to OA in later life. There is evidence to show that 90% of the AKP sufferers had ongoing problems four years later [135] and 94% of the AKP cases continued to experience difficulties for a mean of 8 years following diagnosis [136]. One retrospective study that investigated the association between idiopathic AKP in early adulthood and patellofemoral OA found that patients with patellofemoral OA have described more preceding AKP in their adolescence and early adult years than medial unicompartmental tibiofemoral knee OA cases [137]. A systematic review concluded that there was a paucity of high-quality research evidence regarding the link between idiopathic AKP in younger adults and the subsequent development of patellofemoral OA [5]; however, the authors indicated that the evidence from the retrospective case-control study [137] was reliable as its recall bias was minimal and

choice of a control group was suitable. Therefore, the risk factors for AKP can be regarded as early risk factors for knee OA.

In summary, the direct evidence for an association between childhood obesity measures and adulthood knee pain is rarely explored. The only existing study that explored this association of childhood obesity with adulthood knee pain has not found an independent association of obesity in childhood with adulthood knee pain. This study has a number of potential limitations; these include the use of non-validated knee pain questionnaires and using under weight group as a reference group. These suggest the need for a new study addressing the question using a validated knee pain questionnaire.

1.4.1.3 Early life injury and knee OA

Injury to the joint is a major risk factor for OA. Studies have suggested a consistent association of injury with knee OA in all age groups. Only a few studies have explored the association of childhood injury with the incidence of knee OA in later life. The effect of early life injury on OA seems to be lower than the effect of injury in late adulthood. It is possible that injury is mostly related to an acute surge of symptoms in the joint severely affecting the functional ability which may predispose to the development of OA. In a retrospective study that explored the association between AKP in early adulthood and patellofemoral OA, it was found that the patients in the patellofemoral arthroplasty group had suffered significantly more patellofemoral instability and trauma in their early adulthood than patients with medial unicompartmental arthroplasty [137]. A study exploring the persistence and recurrence of knee pain in preadolescents found that traumatic lower extremity pain had a 50% lower risk for pain recurrence compared with non-traumatic pain indicating a favourable long-term natural course for traumatic pains [138].

Joint hypermobility represents a risk factor for musculoskeletal pain during adolescence, including knee pain. These relationships were strongest in the presence of obesity, which was consistent with a causal pathway whereby joint hypermobility leads to pain at sites exposed to the greatest mechanical forces [139].

1.4.1.4 Early life socioeconomic status and knee OA

Lower socioeconomic status (SES) has been associated with knee pain and knee OA including total knee replacements in adulthood [140]. Macfarlane et al have identified an influence of childhood social status on the adulthood wide spread musculoskeletal pain

including knee pain at 45 years; however, the magnitude of effect of childhood social status on adulthood self-reported pain was less than that of adult social status and was partly explained by poor adult mental health, psychological distress, adverse life events and lifestyle factors [141]. Similarly, there was an independent association of both childhood and current SES with self-reported arthritis [142]. BMI was the most likely mechanism underlying the association of childhood SES with arthritis onset [142].

1.4.1.5 Early life physical activity and knee OA

There is consistent evidence that physical activity improves the pain and physical function in knee OA, and physical activity is included in the guidelines for the nonsurgical management of knee OA [143]. However, the effect of physical activity and fitness on the development and progression of OA is controversial with studies suggesting detrimental [144], beneficial [145] and no effect [146]. The reasons for this controversy are unclear but it may be due to the fact that these studies were mostly retrospective in nature and have not been able to take into account the role of injury, which increases the risk of developing OA and is more common in the physically active people. It is also possible that knee structures behave differently to different physical activity at different life stages. There was weak evidence showing that the healthy articular cartilage in vivo responded positively to physical loading and degenerated cartilage responded negatively [147]. Similarly, in hamsters, physical exercise at a young age had a beneficial effect by enhancing the cartilage maturation, but demonstrated adverse effects on cartilage at a later age with a significant increase in the incidence of OA [148].

In children aged 9-18 years, physical activity was associated with increased cartilage volume both cross-sectionally and longitudinally [149, 150]. In the same study, leg muscle strength was positively associated with cartilage volume and partially mediated the association between physical activity and cartilage volume [151]. Similarly, an interventional study in younger adults (mean age, 25 years) using delayed Gadolinium Enhanced MRI of cartilage (dGEMRIC) scans (for detecting the glycosaminoglycan (GAG) content in the cartilage) showed a positive change of dGEMRIC index over 10 weeks in women who were enrolled in a moderate running programme [152]. Similarly, a RCT evaluated the effect of moderate exercise on GAG content in knee cartilage and found that the exercise group had an increased GAG content compared to controls [153]. There was a strong correlation (r=0.74) between the self-reported physical activity levels and the GAG content in knee cartilage [153]. In contrast, cross-sectional studies of adult triathletes

and middle-aged women found no difference in cartilage volume between those with exercise and without exercise but bone size was generally larger among triathletes than inactive controls [20, 154]. Another cross-sectional study in healthy men found that physical activity was negatively associated with medial tibial cartilage volume [62]. Both these studies were small in sample size and cross-sectional.

A twin study that explored the effect of physical activity on general health found that physical activity reduced the risk of chronic diseases and helped in maintaining life satisfaction; however, there was no association between being active or less active and OA in monozygotic or dizygotic twins [155]. A study done in preadolescents with lower limb pain found that 32% reported pain persistence at 1-year follow-up and 31% reported pain recurrence at 4-year follow-up. Vigorous exercise was a significant predictor of lower limb pain persistence at 1-year follow-up and hypermobility was predictive of pain recurrence at 4 years later [138]. There was evidence to suggest that athletes had higher risks of knee OA features including osteophytes and radiographic OA even in younger adults [156].

In summary, although the evidence for the association between physical activity and fitness with knee structures is controversial, studies in children and young adults indicate a favourable effect of physical activity and performance on knee structures. There are no reports so far to explore the independent associations of physical performance measures in childhood with adulthood knee structures.

1.5 Summary

About 8% of Australians are affected by OA. By 2050, it is projected that the prevalence of OA will be 11% of the population. OA is a leading source of health expenditure on arthritis, accounting just under half of total allocated expenditure on arthritis in 2007. There was a 54% rise in total knee replacements for OA from 2002-03 to 2011-12. There are no disease-modifying treatments available for OA. There is an urgent need for identifying the modifiable risk factors for this disease. Identifying these factors in early life itself can help to prevent or delay the development of OA in later life. The following chapters investigate the roles of childhood and early adulthood factors such as obesity, physical activity and physical performance in knee joint health assessed using knee MRI. A 25-year childhood follow-up cohort study was used to explore these questions. Novel analyses have been performed examining the association of physical activity and cholesterol measures with BMLs. The specific research questions that directed this work are described in the following chapter.

Chapter 2 - Research questions

The following research questions were determined in a population-based sample of young adults aged 31-41 years:

1) Were physical performance measures including physical work capacity at 170 heart beats per minute (PWC_{170}), time taken to complete the long-run and short-run, number of sit-ups completed and leg muscle strength in childhood associated with adult knee tibial cartilage volume and tibial bone area 25 years later?
   Hypothesis: PWC_{170}, time taken to complete the long-run and short-run, number of sit-ups completed and leg muscle strength in childhood are positively associated with adult knee tibial cartilage volume and tibial bone area.

2) Were overweight measures in childhood associated with knee pain, stiffness and physical dysfunction among adults 25 years later?
   Hypothesis: Overweight measures in childhood are positively associated with knee pain, stiffness and physical dysfunction among adults 25 years later.

3) Were body composition, hormonal and inflammatory factors associated with tibial cartilage volume, and did these factors explain sex difference in tibial cartilage volume in young adults?
   Hypotheses: 1) Lean mass and sex hormone binding globulins (SHBG) are positively associated with tibial cartilage volume. 2) Fat mass, testosterone, C-reactive protein (CRP) and fibrinogen are negatively associated with tibial cartilage volume. 3) Body composition and inflammatory factors explain the sex difference in tibial cartilage volume in young adults.

4) Were physical activity and physical performance measures assessed 5 years prior associated with tibial cartilage volume and bone area in young adults?
   Hypothesis: Physical activity and physical performance measures assessed 5 years prior are positively associated with tibial cartilage volume and bone area in young adults.

5) What were the environmental (physical activity), structural (cartilage defects, meniscal lesions) and clinical (knee pain, knee dysfunction) correlates of bone marrow lesions (BMLs) in younger adults, and were cholesterol levels measured 5 years prior associated with current BMLs in young adults?

Hypotheses: 1) BMLs in young adults are positively associated with structural (cartilage defects and meniscal lesions) and clinical (knee pain, knee dysfunction) features of knee osteoarthritis. 2) Physical activity in young adults is negatively associated with BMLs, 3) Cholesterol levels measured 5 years prior are associated (high-density lipoprotein (HDL) negatively and low-density lipoprotein (LDL) and total cholesterol (TCH) positively with current BMLs in young adults.

Chapter 3 - Methodology

3.1 Prelude

This thesis arose from the analyses of Childhood Determinants of Adult Health (CDAH) Knee Cartilage study population, and a number of outcome factors, study factors, and covariates have been utilised. This chapter describes the CDAH Knee Cartilage study population and its design, as well as the protocols for measurement of factors which are common to multiple chapters in this thesis. Additional factors that are unique to each chapter are described in more detail within the methodology section of each of the subsequent chapters.

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

3.2 Study population and design

The work in this thesis was conducted as part of the CDAH Knee Cartilage study, a follow-up of a sub-sample of participants who completed the Childhood Determinants of Adult Health (CDAH) study in 2004 to 2006. CDAH study was a 20-year follow-up of the Australian Schools Health and Fitness Survey (ASHFS) of 1985. The flowchart showing selection of the participants for the current CDAH Knee Cartilage study from previous studies are presented below (Figure 3.1).

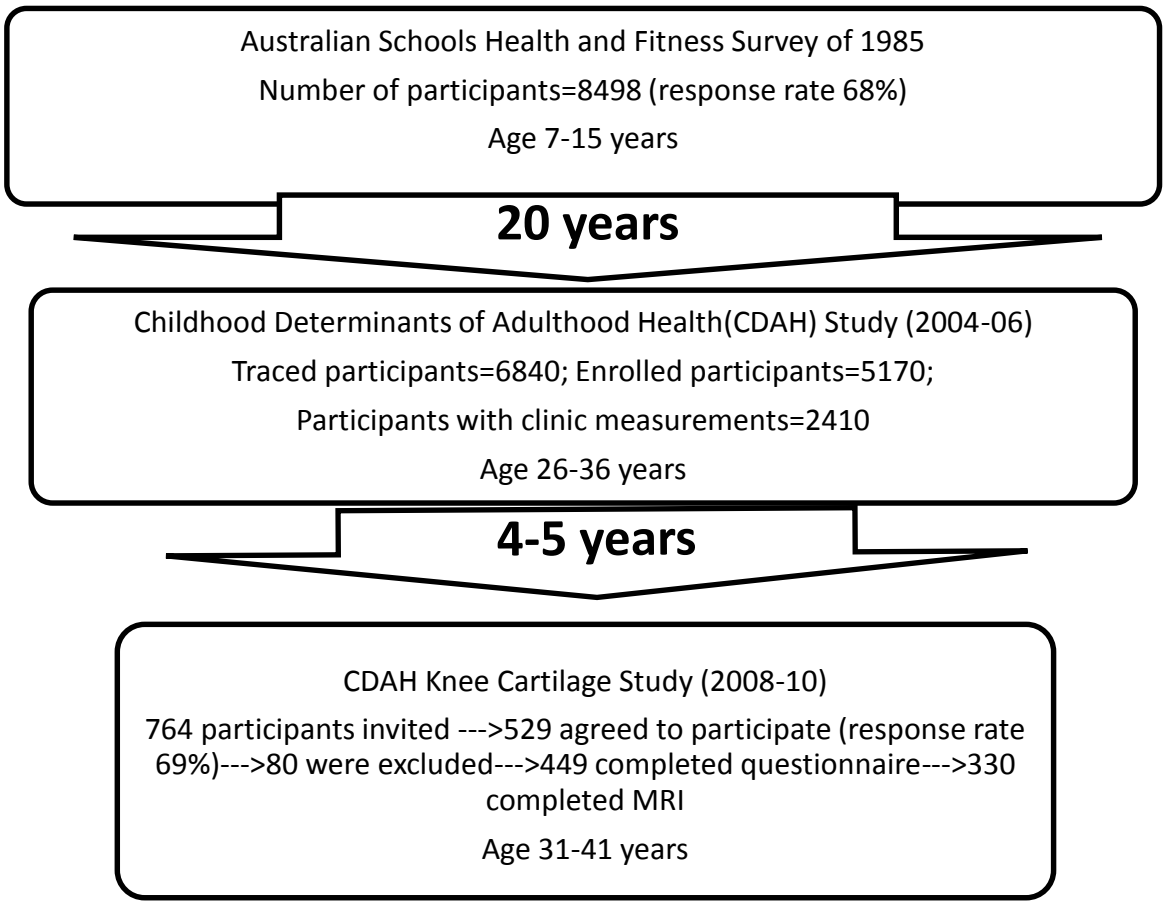


Figure 3.1. Flowchart showing selection of the participants for the CDAH Knee Cartilage study from previous studies

3.3 ASHFS

ASHFS examined the health and fitness of 8498 Australian children aged 7-15 years. School children were selected randomly from 109 schools Australia-wide. Details of the 1985 sampling strategy have been described previously [157]. Briefly, the eligible schools were selected based on their ability to provide groups of ten students in required age and sex categories. Eligible schools were listed in ascending postcode order to ensure a wide geographical distribution. 109 schools were selected from postcode with a random start and constant interval. Then the students were categorised by age and sex based on school enrolment information, with 15 students from each age/sex category being systematically selected. The State Directors General of Education granted approval to contact schools, and all children provided assent and parents provided written informed consent. Among 12,578 students who were aged 7-15 years and invited to participate in the ASHFS, 8498 participated, representing an overall response rate of 67.5%.

All the data collection team members were trained from the central study-co-ordinator. The trained assessors at the participating schools completed a range of field, technical, laboratory and blood tests as well as questionnaire. All participants completed height, weight, sit-ups, a 50-m short-run and a 1.6-km long-run. Sub-samples were used for more demanding technical tests. Only 9, 12 and 15-year-olds completed muscular strength tests using dynamometers and cardiorespiratory fitness measures using the physical work capacity 170 bicycle ergometer test.

3.3.1 Anthropometrics

Weight was measured to the nearest 0.5 kg (with shoes, socks, and bulky clothing removed). Height was measured to the nearest 0.1 cm (with shoes and socks removed) using a stadiometer. Body mass index (BMI) was calculated as kilograms per square meter (kg/m^2). Healthy weight was defined as a BMI less than the internationally accepted age- and sex specific cutpoints for overweight and obesity developed by the International Obesity Task Force (IOTF) [158]. The cutpoints of BMI used in our study for defining overweight ranged from 17.75 kg/m^2 (7 year old girl) to 24.17 kg/m^2 (15 year old girl). Similarly, the cutpoints of BMI for defining obesity ranged from 20.51 kg/m^2 (7 year old girl) to 29.11 kg/m^2 (15 year old girl). These cutpoints are also recommended by the National Health and Medical Research Council (NHMRC) for the use in population and clinical research in children [159].

3.3.2 Physical performance measures

3.3.2.1 Long-run

The 1.6-km run was assessed on a 400- or 200-m grass track. Children were instructed on the number of laps to be completed and that the technique best used for running the distance is to maintain a steady pace for the start and middle of the distance, and that they could increase speed at the end of the distance if they still felt comfortable and had the endurance. A score sheet was used to check off the number of laps completed by each subject. Timekeepers used stop watches and recorded subjects' times (min:s) as they passed the finish line.

3.3.2.2 Short-run

The 50-m sprint was run on a straight, level, 50-m track at right angles to the wind direction. Subjects were given a thorough warm-up with stretching and light jogging before testing. The subjects started behind the staring line in a standing position. There was only one trial, so the subjects were instructed to do their best and run as fast as possible until well past the finish line. Subjects ran in small groups with as many runners as there were timekeepers. The time was measured to the nearest 1/100th of a second.

3.3.2.3 Leg muscle strength

Leg muscle strength was measured to the nearest 1.0 kg using an isometric leg muscle strength dynamometer (TTM Muscle Meter, Tokyo, Japan) in a sample of ASHFS participants who were at 9, 12 and 15 years of age. Subjects were asked to stand on the dynamometer with a straight back, flat against the wall, holding the hand bar with an overhand grip. Knees were flexed until an angle of 115° was obtained from the neutral angle of full extension (depend on participant range of motion), at which the bar was attached to the dynamometer by a chain. Subjects kept a firm grip on the bar and pulled upward using only their legs, and keeping the back and neck straight, as far as possible. Subjects were instructed in each technique before testing and verbal encouragement was given until a maximal contraction was achieved. Two trials were recorded, with the mean score taken as the criterion value for leg strength. This test examines isometric strength of the whole legs, but predominantly of the quadriceps and hip extensors. The high correlation of 0.78 between this test and a specific test of quadriceps function was reported

(S Foley, unpublished data, 2007). A repeatability estimate (intraclass correlation coefficient) was not assessed in this population but has previously been reported as 0.91[150].

3.3.2.4 Sit-ups

Sit-ups were performed on a level floor area with a mat or carpet. The student was asked to lie on the floor with knees bent to 140° and heels on the ground and hands with fingers extended on the front of the thigh. The student was asked to curl-up the trunk and move the fingers up the thigh to the level of patella in time to a cadence sound tape. This position was maintained for one second before returning to the original position with back and head touching the ground. The test was continued for 5 minutes to a maximum of 100 sit-ups. A cadence of 20 sit-ups per minute was maintained and the execution of the test was supervised.

3.3.2.5 Physical work capacity at 170 beats per minute

Cardiorespiratory fitness in ASHFS was estimated based on physical work capacity at a heart rate of 170 beats/min (PWC_{170}). This was assessed using a bicycle ergometer (Monark exercise bicycle) [160]. Subjects were asked to cycle at a constant 60 revolutions per minute for 3 minutes each at three successively increasing but submaximal workloads. Heart rate was recorded at 1-min intervals at each workload using an electronic heart rate monitor. The target heart rate was 115-130 in workload 1, 130-145 in workload 2 and 145-160 in workload 3. Work capacity at 170 beats/min was assessed by linear regression with extrapolation of the line of best fit to a heart rate of 170 beats/min. The PWC_{170} was not considered a technically adequate measure unless subjects had spent a minimum of 2 min at each workload. Repeatability was not assessed in our subjects but has previously been reported as 0.92 [161].

VO_2 max is a universally accepted laboratory measure of cardiovascular fitness but is unsuitable for fieldwork, especially for a nationwide study such as the ASHFS. Furthermore, motivating children to achieve maximal effort is highly problematic. As a result, the most popular methodologies to predict VO_2 max in children have used submaximal bicycle ergometry to determine the physical work capacity. PWC_{170} is safer, less expensive, more portable, and does not require the same level of motivation as a maximal test [162]. In the ASHFS, 261 subjects also completed VO_2 max test. The correlation between PWC_{170} and VO_2 max was found to be 0.83 [162].

3.4 CDAH study

Participants in 1985 ASHFS were contacted for the conduct of CDAH study during 2004-2006 (approximately 20 years after ASHFS). A mixed approach was used for tracing participants which included searching current and historical electoral rolls, electronic telephone listings, the National Death Index, and contact with located classmates. Potential participants were sent an information package which contained an invitation letter, project information, a consent form and an enrolment questionnaire. Of the original 8498, 6849 (80.6%) were successfully traced, and 5,170 individuals agreed to participate in the CDAH study and completed an enrolment questionnaire (61% of total sample, 76% of those traced) between 2001 and 2004. However, only a total of 2410 participants (52% female, 48% male) attended one of 34 study clinics in major cities and regional centres around Australia for extensive physical measurements in 2004-6.

Anthropometrics, blood biochemistry and physical performance measures including cardiorespiratory fitness and muscular strength were measured during the clinic visit by trained staff and a pedometer was issued before participants left the clinic. Geographical Information Systems (GIS) software was used to map participants' current postcode (Figure 3.2) in order to determine clinic locations and to facilitate participants' attendance.

The aims of the CDAH study were to examine the contribution of childhood factors to the risk of adult cardio-metabolic diseases, mental and musculoskeletal health. All participants provided written informed consent and the study was approved by the Southern Tasmania Health and Medical Human Research Ethics Committee (Ethics Approval Number: H0008152)

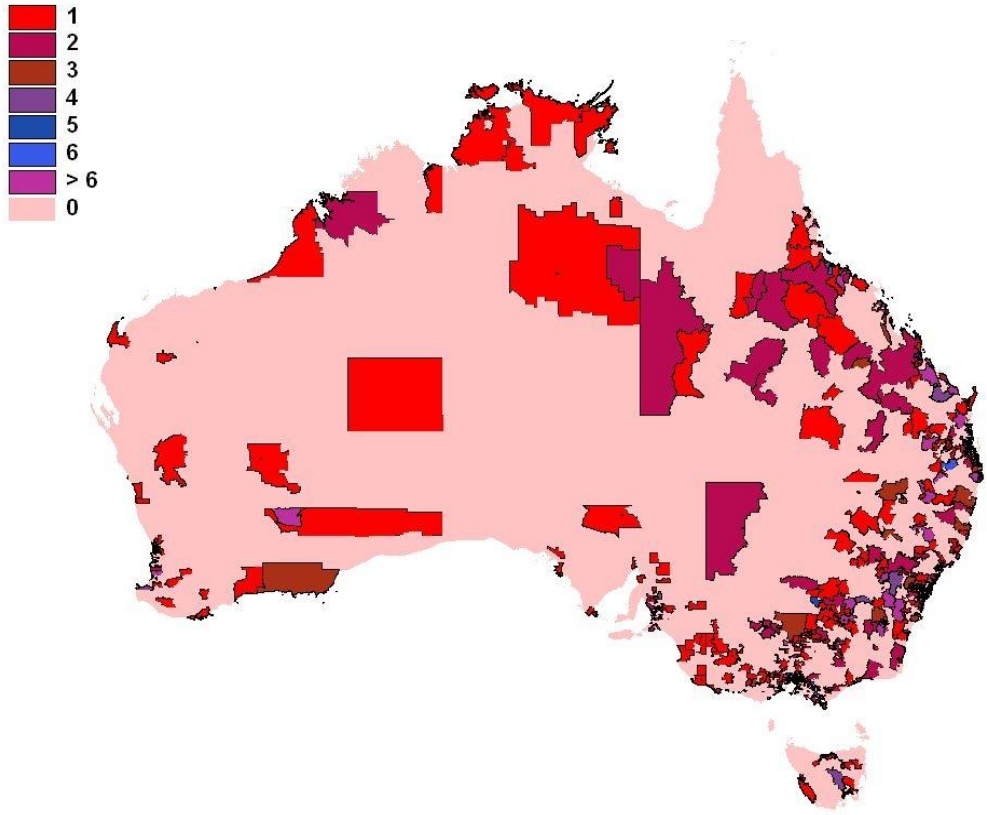


Figure 3.2. Distribution of participants across Australia in the CDAH study at follow-up (2004-2006). The key represents the number of participants tested in each postcode of Australia

3.4.1 Anthropometrics

Height in centimetres was measured using a Leicester stadiometer to one decimal point (0.1 cm) with participant in bare feet and any obstructive headwear removed. Weight was measured in kilograms to one decimal place (0.1 kg) using calibrated digital Heine scales with participants' shoes and heavy cloths removed. BMI was calculated using the formula: BMI=weight (kg)/(height (m))^2.

Waist circumference was measured using a Lufkin Thinline Executive 2-meter nonstretch steel tape measure (W606PM) in light clothing. Waist girth was taken at the level of the narrowest point between the lower costal border and the iliac crest. The same qualified anthropometrist (technician qualification Level 1, International Society for the Advancement of Kinanthropometry) trained a staff member in each state to conduct the adiposity measures.

Skin-fold measurements were also taken during the CDAH study by the same technicians, who were trained in accordance with the international standards of

anthropometric assessment. They used anatomical landmarks to locate and measure skin-folds at tricep, bicep, subscapular and subiliac regions to the nearest 0.1 mm, using Slim Guide Calipers (SPRI Products, Libertyville, IL). Estimate of percent body fat was derived from the sum of skin-folds according to published equations for adults [163] and lean body mass (LBM) and fat mass was calculated using weight (kg) (LBM = weight – ((fat% × weight)/100)).

3.4.2 Physical performance measures

3.4.2.1 Leg muscle strength

Leg muscle strength was measured to the nearest 1.0 kg using dynamometers (TTM Muscle Meter, Tokyo, Japan). Measurement protocol was similar to the childhood measurement. Briefly, subjects were asked to stand on leg dynamometer with a straight back, leaning on the wall and holding the bar hand with an overhand grip. Knees were flexed to 115° at which the bar was attached to the dynamometer by a chain and the subject was asked to pull the chain by straightening the knee but keeping back and neck straight [162, 164].

3.4.2.2 Long jump

Standing long jump was measured by asking subject to stand on the gym mat with toes behind the line and with feet slightly apart. A two-feet take-off and landing was used, with the subject swinging arms and bending knees to provide the drive for the jump. Each subject was allowed two trials, with the best score counted. The jump was repeated if the subject fell back or used a step at take-off. The landing point at the closest part of the heel to the starting line was marked and the distance to the starting line was measured.

3.4.2.3 PWC_{170}

Cardiorespiratory fitness was estimated using a submaximal graded exercise test to estimate physical work capacity at a heart rate of 170 beats/min. To be consistent with baseline cardiorespiratory measurement, the test followed standardized procedures [160]. Briefly, after a short warm-up, subjects were asked to cycle on a Monark cycle ergometer (model 828E, Monark Exercise AB, Sweden) for 12 minutes at a constant 60 revolutions per minute. The workload was increased at the end of every four-minute period, so that the

heart rate achieved by the participant at the end of the first, second and third workloads were ≥115, ≥130, ≥145 beat per minute, respectively. Steady-state heart rate was recorded in the last 15 seconds of each workload using a wireless polar heart rate monitor (Polar Electro Oy, Finland). PWC_{170} was assessed by linear regression with extrapolation of the line of best fit to a heart rate of 170 beats/min. Repeatability and ICC are mentioned in the previous description of PWC_{170}.

3.4.3 Physical activity

Participants completed the self-administered long version of the International Physical Activity Questionnaire (IPAQ-L) to estimate their physical activity in the last week. The IPAQ is an internationally recognised survey developed by an expert committee for use in adults aged 18-65 years that has an acceptable level of validity and reliability [165]. Physical activity for leisure, transport, work and domestic purposes in the past week were assessed. Frequency, duration and intensity of various domains of physical activities were considered including leisure and work related physical activity. Participants were asked to report those activities that they participated in for at least 10 minutes duration and examples were provided. The total weekly minutes spent in each domain of physical activity was calculated by multiplying the duration and frequency of each activity within each domain of physical activity. Total physical activity was calculated to represent minutes per week of vigorous physical activity, moderate physical activity and walking from all domains of physical activity. Regular participation is a key concept included in current public health guidelines for physical activity [166]. Therefore, both the total volume and the number of day/sessions are included in the IPAQ analysis algorithms. Three levels of physical activity ‘low’, ‘moderate’, and ‘high’ were developed from the IPAQ-L according to the guidelines (https://sites.google.com/site/theipaq/scoring-protocol; Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire). The IPAQ demonstrates very good levels of repeatability and fair to moderate validity when compared to accelerometer data [165].

3.4.4 Blood collection and biochemistry

32 ml of venous blood was collected from the antecubital vein of reclined (seated or lying) participants after an overnight fast of at least 8 hours. All samples were centrifuged within two hours of venipuncture and stored upright in a fridge or ice container and held at approximately 4 °C. At the completion of each clinic day, all samples were

sealed in an insulated container with cold packs and dispatched via overnight courier to the central analysing laboratory (MedVet, Adelaide, South Australia) where assays were completed. Temperature during transportation ranged from 0.7 °C to 3.6 °C (mean temperature, 2.6 °C). The analytical laboratory met the criteria for precision accuracy as specified for standardisation by the WHO Centre for Reference and Research in Blood Lipids (Centers for Disease Control and Prevention, Atlanta, Georgia).

3.4.4.1 Hormone measurements

Total testosterone concentrations and sex hormone binding globulin (SHBG) were estimated in female participants who were not using oral contraceptives at a specialised reproductive endocrine laboratory (the Queen Elizabeth Hospital in South Australia). Total testosterone concentrations were estimated by radioimmunoassay (RIA) developed by Repromed Laboratory (Dulwich, South Australia), which is sensitive for lower levels of testosterone down to 347 pmol/L. SHBG was measured using a non-competitive liquid-phase immunoradiometric assay (SHBG-IRMA kit, Orion Diagnostica, Espoo, Finland). For testosterone, the intra- and inter- assay coefficients of variation (CVs) were 6% and 15%, respectively. For SHBG, the inter- and intra- assay CVs were 8.6% and 15.4%, respectively [167]. Free androgen index (FAI), the active testosterone in the blood, was calculated as: testosterone (nmol/L)/SHBG (nmol/L).

3.4.4.2 CRP and fibrinogen

Serum CRP was determined using an automated analyzer (Olympus AU5400) and a highly sensitive turbidimetric immunoassay kit (Olympus System CRP Latex reagent, Olympus Life and Material Science Europa GmbH, Ireland) by MedVet (Institute of Medical and Veterinary Science, Adelaide, South Australia). Plasma concentration of fibrinogen was determined by the Clauss clotting method using the STA automated coagulation analyser (STA-Fibrinogen reagent, Diagnostica Stago, Manufactured in Paris, France Distributed from Parsippany, NJ, USA).

3.4.4.3 Cholesterol measures

Samples for lipid assays were stored in white-top serum tubes and allowed to coagulate for 15 minutes at room temperature prior to centrifugation. Serum total cholesterol and HDL cholesterol concentrations were determined enzymatically (Olympus

AU5400 automated analyzer, Olympus Optical, Tokyo, Japan) and the Lipid Research Clinic procedures were followed. LDL cholesterol concentration was calculated using the Friedewald formula [168]. LDL Cholesterol = total cholesterol – (triglycerides／ 2.2 + HDL cholesterol). The inter-assay CV ranged from 2.1 to 2.8% for total cholesterol, 4.2 to 5.4% for HDL cholesterol and 3.0 to 4.6% for triglycerides. Duplicate aliquots from a single blood draw were collected from the first participant scheduled for each clinic day (N=108) to examine measurement errors associated with collection, processing, and analysis of blood samples. All duplicate assays were conducted by technicians blinded to the first-run results. The CVs were 1.6% for total cholesterol, 2.8% for HDL cholesterol and 2.6% for triglycerides.

3.5 CDAH Knee Cartilage study

CDAH Knee Cartilage study was designed to investigate the effects of childhood and adulthood physical activity, fitness and fatness on adult knee joint structures. This study was designed following the earlier findings from the CDAH study that childhood physical fitness and fatness were associated with adult bone mineral density (BMD) [162]. Figure 3.3 provides an overview of participant recruitment and withdrawal during the study period. This cohort consisted of both males and females aged between 31 and 41 years (mean ± standard deviation (SD) age = 36 ± 4 years), selected from the CDAH study. The CDAH Knee Cartilage study was conducted from April 2008 to December 2010 (approximately 5 years after CDAH study and 15 years after ASHFS). The CDAH study participants (n=764) living in Melbourne and Sydney were contacted and invited to participate in the CDAH Knee Cartilage study by sending an invitation letter (Appendix 1). Eligibility criteria were assessed in subjects who agreed to participate (n=529, response rate 69%). Exclusion criteria included the following: being pregnant; having diseases that may affect knee cartilage such as rheumatoid arthritis; having a contraindication for MRI including claustrophobia.

Eighty subjects were excluded, and the remaining 449 subjects were asked to complete a short computer assisted telephone interview (CATI) (Appendix 3). CATI included questionnaires on physical activity (short IPAQ), knee pain, stiffness and physical dysfunction questionnaire (Western Ontario and McMaster University Osteoarthritis Index (WOMAC)), smoking status, and history of knee injury.

Subjects were then requested to have an MRI scan at Epworth Hospital in Melbourne or North Shore Private Hospital in Sydney. Some participants (n=119) did not

undergo MRI after enrolling in the study due to the long distance that they would have needed to travel for MRI, work or family commitments, moving interstate, becoming pregnant by the time of MRI, or changing their mind. This study was approved by the Southern Tasmania Health and Medical Human Research Ethics Committee, the Monash University Human Research Ethics Committee and the Northern Sydney and Central Coast Area Human Research Ethics Committee (Ethics Approval Number: H0009828), and all participants provided written informed consent (Appendix 2).

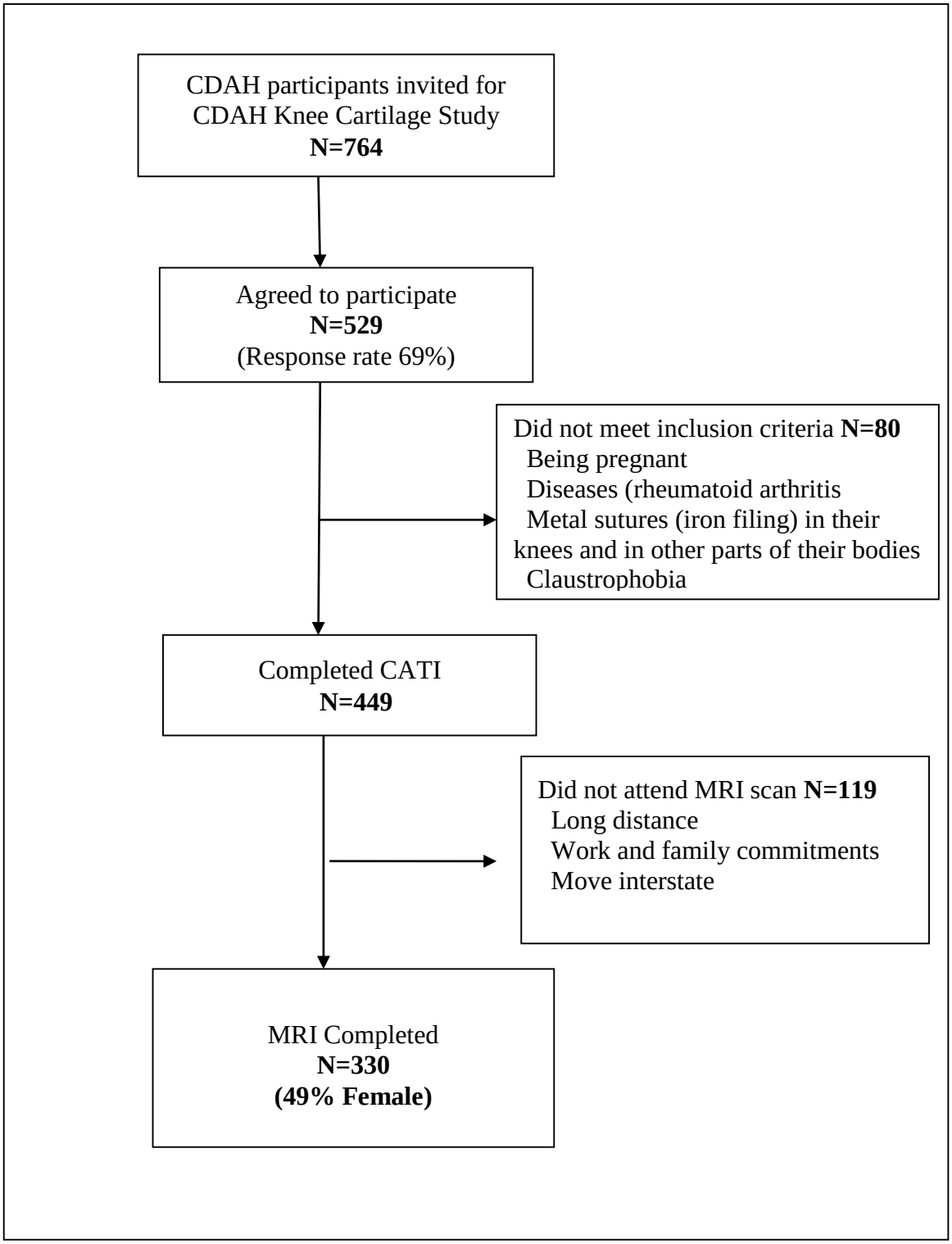


Figure 3.3. Flow chart describing recruitment, participation rates, and withdrawal reasons for CDAH Knee Cartilage study participants.

3.5.1 Anthropometric measurements

Weight was measured to the nearest 0.1 kg in CDAH Knee Cartilage study with shoes, socks, and bulky clothing removed at the time of MRI. Height was measured to the nearest 0.1 cm (with shoes and socks removed) using a stadiometer in CDAH study. Body mass index (BMI) was calculated as kilograms of weight per square metre of height.

3.5.2 Knee symptom assessment

Knee symptoms during the past 30 days were assessed using the Western Ontario and McMaster University osteoarthritis index (WOMAC) scale in CDAH Knee Cartilage study. Participants were asked about their knee pain, stiffness and physical dysfunction status during computer assisted telephone interview (Question 25-27, Appendix 3). There are 5 questions (subscales) in WOMAC knee pain, 2 subscales in WOMAC stiffness and 17 subscales in WOMAC dysfunction. Each question was graded on a scale of 0-9, where 0 indicated no symptoms and 9 indicated the maximum intensity of the symptoms. Total WOMAC scores were calculated by adding the scores of 5 subscales in WOMAC knee pain (0-45), 2 subscales in WOMAC stiffness (0-18) and 17 subscales in WOMAC dysfunction (0-153). The WOMAC is an established scale for OA research and is also validated for responsiveness of knee complaints in young population without OA [169]. The WOMAC index has good test–retest reliability, with values of 0.68 for the pain scale and 0.48 for the function scale [170], and has demonstrated convergent construct validity over numerous impairments[171]. Responsiveness of the WOMAC is variable depending on the intervention measured [171], as expected.

3.5.3 Physical activity measurements

Subjects in the CDAH Knee Cartilage study completed a short version IPAQ to estimate their physical activity in the last week. Participants were asked to report those activities that they participated in for at least 10 minutes duration using CATI (Question 15-21, Appendix 3). Physical activities were calculated to represent minutes per week of vigorous physical activity, moderate physical activity, walking and total physical activity as described in long IPAQ (CDAH study). The short version of IPAQ also demonstrates good levels of repeatability and fair to moderate validity when compared to accelerometer data [165].

3.5.4 Knee injury status

History of knee injury or surgery was not collected in childhood ASHFS study and therefore, telephone interview in CDAH Knee Cartilage study included history of knee injury in childhood and adulthood. Knee surgery information was also collected in CDAH Knee Cartilage study. This information was recorded in response to the question "Have you had a knee injury requiring non-weight bearing treatment for more than 24 hours or surgery?" Details of the nature of injury and surgery were also collected for the evaluation.

3.5.5 Magnetic resonance imaging

MRI of the dominant knee was acquired from two hospitals, which used the same type of machine (General Electric Medical Systems, Milwaukee, WI, USA). Knees were imaged in the sagittal plane on a 1.5 T whole body magnetic resonance unit with use of a commercial transmit-receive extremity coil. The following image sequence was used: 1) T1-weighted fat saturation 3D spoiled gradient recall acquisition in the steady state; flip angle 55 degrees; repetition time 58 msecs; echo time 12 msec; field of view 16 cm; 60 partitions; 512 x 512 matrix; acquisition time 11 min 56 sec; one acquisition. Sagittal images were obtained at a partition thickness of 1.5 mm and an in-plane resolution of 0.31 x 0.31 (512 x 512 pixels). 2) proton density-weighted fat saturated 2D fast spin echo coronal images at a partition thickness of 3.3mm and an in-plane resolution of 0.31 x 0.31 (512 x 512 pixels).

Ten volunteers had MRI scans performed at both hospitals' MRI machines to determine the variation between machines. The CVs for cartilage volume measurements were 9.5% (lateral tibial) and 16.2% (medial tibial), and for bone area measurements were 5.9% (lateral tibial) and 5.7% (medial tibial). Bland-Altman plots were used to examine the observed difference between these machines and this difference was dependent on the magnitude of the reading. Based on these 10 volunteers, we calculated the correction equations for cartilage volume and bone area using the slope and intercept from a linear regression model where the result from one machine was regressed on the result from other machine. The corrections were performed by keeping the values of one scan location (Melbourne) as they were and then changing the values of another scan location (Sydney) by multiplying the beta (0.88, 0.94, 0.66, 1.34, respectively, for medial and lateral tibial cartilage volume, and medial and lateral tibial bone area) of linear regression and adding the constant (0.53, 0.55, 6.11, -2.97, respectively, for medial and lateral tibial cartilage volume, and medial and lateral tibial bone area).

3.5.6 Cartilage volume

Knee tibial and patellar cartilage volumes were assessed on T1-weighted MR images by means of 3D image processing on an independent work station using the software program OsiriX (Geneva, Switzerland). Individual plates of tibial cartilage volume (medial and lateral) and patella cartilage volume were isolated by manually drawing disarticulation contours around the cartilage boundaries on a section-by-section basis (out of 55-65 slides). These data were then re-sampled by means of bilinear and cubic interpolation (area of 312 × 312 μm^2 and thickness of 1.5 mm, continuous sections) for the final 3D rendering as previously described [172]. The CVs for cartilage volume measures were 2.1-2.6% [7]. Total tibial cartilage volume was calculated as the sum of medial and lateral tibial cartilage volume.

3.5.7 Tibial bone area

Knee tibial plateau bone area was assessed on T1-weighted MR images and defined as the cross-sectional surface area of the tibial plateau. The bone area of medial and lateral tibial plateau was measured manually on the three reformatted T1-weighted MR images closest to tibial cartilage in the axial plane as describe in the previous studies [7, 69, 173]. The border of the tibial articular surface was manually marked and the area was recorded. The slice thickness on the axial images was 0.625 mm. The CVs for these measures were 2.2-2.6% [7]. Total tibial bone area was calculated as the sum of medial and lateral area.

3.5.8 Bone marrow lesions

Subchondral bone marrow lesions (BMLs) were assessed on coronal proton density-weighted images using OsiriX software (University of Geneva, Geneva, Switzerland) and were defined as areas of increased signal adjacent to the subchondral bone at the medial tibial, medial femoral, lateral tibial, and lateral femoral sites. BMLs were scored using an ordinal scoring system and an areal scoring system which we used previously described [24]. Each BML was scored on the basis of lesion size. Subjects with no BMLs were scored as grade 0 and then the subjects with BMLs were graded according to the percentage of area of occupancy of BML in each compartment: grade 1: ≤25% of area; grade 2: >25%<50%; grade 3: >50%. The BML with the highest score was used if more than one lesion was present at the same site. The ICCs were 0.94, 1.00, 0.89, and

0.96 at the medial tibial, medial femoral, lateral tibial, and lateral femoral sites, respectively [24].

3.5.9 Cartilage defects

Cartilage defects were measured using both proton density coronal images and T1-weighted sagittal images at the medial tibial, medial femoral, lateral tibial, and lateral femoral sites, as previously described [8]. Grade 0 indicated a normal cartilage, grade 1 indicated focal blistering and low (T1-weighted) or high (proton density-weighted) signal intensity area with intact surface/bottom. Grade 2 indicated a loss of thickness of less than 50% on surface/bottom of the cartilage. Grade 3 represented a deep ulceration with loss of thickness >50%, and grade 4 indicated a full-thickness chondral wear with exposure of subchondral bone. A prevalent cartilage defect was defined as a cartilage defect score of ≥2 at any site within that compartment. The cartilage was considered to be normal if the band of intermediate signal intensity had a uniform thickness. The highest score was used if more than one defect was present on the same site. Intraobserver repeatability was assessed in 50 subjects with an interval of at least one week between the two measurements. ICCs were 0.93, 0.92, 0.95, and 0.80 at the medial tibia, medial femur, lateral tibia, and lateral femur, respectively.

3.5.10 Meniscal pathology

Meniscal tear was graded in medial and lateral menisci separately based on a combined Whole-Organ Magnetic Resonance Imaging Score (WORMS) scoring system [174] from grade 0 to 2 using proton density-weighted coronal and T1- weighted sagittal images. Grade 0 was a fully normal intact meniscus. Grade 1 indicated a non-displaced tear (scored as grade 1 and 2 by WORMS). Grade 2 indicated a displaced tear or maceration (scored as grade 3 and 4 by WORMS).

Meniscal extrusion was recorded on the medial and lateral menisci and was graded from grade 0 to 2 based on the proton density-weighted coronal images as published before [175]. Grade 0 was an intact meniscus without any degree of extrusion. Grade 1 indicated a partially displaced meniscus with respect to tibia and Grade 2 represented a completely displaced meniscus. Meniscal lesions were defined as any meniscal tear or meniscal extrusion in the knee.

3.6 Summary of outcome factors, study factors, and covariates

Table 3.1 summarises the variables used in each chapter of this thesis.

Table 3.1. Summary of outcome factors, study factors, and covariates used in this thesis

Chapter	Outcome factors* (Adulthood)	Study factors* (Childhood and adulthood)	Covariates
4	Tibial cartilage volume Tibial bone area	Childhood physical performance measures (PWC_{170}, long-run, short-run, sit-ups, leg strength)	Age, sex, BMI, duration of follow-up, knee injury, tibial bone area
5	WOMAC knee pain, stiffness, physical dysfunction	Childhood obesity measures (weight, BMI and overweight)	Age, sex, adulthood BMI, duration of follow-up, knee injury, smoking
6	Tibial cartilage volume	Adulthood obesity measures (lean mass, fat mass), inflammatory markers (fibrinogen, CRP), sex hormones (testosterone, SHBG)	Age, sex, height, knee injury, tibial bone area
7	Tibial cartilage volume Tibial bone area	Adulthood physical performance measures (PWC_{170}, long-jump, leg strength) Adulthood physical activity measures (long IPAQ)	Age, sex, BMI, knee injury, tibial bone area
8	BMLs	Adulthood physical activity measures (short IPAQ) Adulthood Cholesterol levels (total cholesterol, LDL cholesterol, HDL cholesterol)	Age, sex, BMI, knee injury

BMI: body mass index; PWC_{170}: physical work capacity at 170 beats/min;
WOMAC: Western Ontario McMaster University osteoarthritis index;
IPAQ: international physical activity questionnaire;
CRP: C-reactive protein; SHGB: sex hormone binding globulin;
LDL: low-density lipoprotein; HDL: high-density lipoprotein
*Measurement protocol described in "Materials and Methods" section of the relevant chapter.

3.7 Sample size and role of the candidate in the CDAH Knee Cartilage study

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

Whilst the candidate was involved in the MRI data acquisition during candidature, the other data acquisition was completed prior to the candidature by a number of other CDAH Knee Cartilage study staff, including Changhai Ding, Graeme Jones, Flavia Cicuttini, Lyn March, Marita Dalton, Liz O'Loughlin and Judy Hankin. The candidate gratefully acknowledges the assistance of Rob Warren in acquiring the cartilage volume and bone area data and Marita Dalton and Tim Albion for managing the database. The candidate performed all other data cleaning and other MRI outcome measures.

3.8 Ethical considerations

In ASHFS consent from both parent and child were obtained. Children provided the assent and parents provided the informed consent. ASHFS was approved by the State Directors General of Education.

In CDAH study, all participants provided written informed consent and the study was approved by the Southern Tasmania Health and Medical Human Research Ethics Committee (Ethics Approval Number: H0008152)

All procedures in CDAH Knee Cartilage study were approved by the Southern Tasmanian Health and Medical Human Research Ethics Committee, the Monash University Human Research Ethics Committee and the Northern Sydney and Central Coast Area Human Research Ethics Committee (Ethics Approval Number: H0009828). Written informed consent (Appendix 2) was obtained from all participants prior to enrolment in the study.

3.9 Statistical analysis

T-tests and chi-squared tests were used to compare differences in means and proportions as appropriate. Linear regression was performed to describe the association between cartilage volume and /or bone area with childhood and adulthood factors. Log binomial regression was performed to describe the association between categorical outcome measures such as presence of knee pain, stiffness, physical dysfunction and BML with childhood and adulthood factors. A *P* value less than 0.05 (two-tailed) was considered statistically significant. More detailed description of statistical analyses performed are presented in the relevant chapters. All statistical analyses were performed on SPSS 19 for Mac (SPSS Inc., Chicago, USA).

Chapter 4 - Childhood physical performance measures predict adulthood knee cartilage volume and bone area: a 25-year cohort study

4.1 Introduction

Osteoarthritis (OA) is the most common joint disorder in adults. It is considered to be a disease of the whole organ including subchondral bone and cartilage. OA is an irreversible disease in most cases, and the ideal treatment would preserve the articular cartilage from further degradation. The knee is the leading site of OA [60].

The promotion of physical activity and fitness is a major public health initiative in western society. It is widely recommended to patients with knee and hip OA for improving their symptoms although the effect of physical activity and fitness on the development and progression of OA is controversial [95, 99, 176-178]. Some studies focusing on physical performance measures (PPMs) such as muscle strength and physical fitness have found a protective effect on knee structures; [75, 91, 92] however, a randomised controlled trial was designed to evaluate strength training programme over 30-month in an older population and reported that strength training had no effect in those with radiographic OA, but significantly increased the rate of joint space narrowing (JSN) in those with normal radiographs at baseline [99]. Physical fitness significantly but modestly correlates with physical activity [179]. A systematic review found limited evidence regarding the association between physical activity and cartilage volume among adults [86].

In younger populations, there is no study demonstrating that PPMs are associated with knee structures but evidence has suggested a beneficial effect of physical activity [152]. A small cross-sectional study of adult triathletes found no difference in cartilage volume or thickness but bone size was larger among triathletes than inactive volunteers [20]. We reported that physical activity was associated with increased cartilage volume among randomly selected healthy children [149, 150]. While physical activity reflects a behavior, physical performance is operationalized as several measurable health-related phenotypes including cardiorespiratory fitness and muscle performance which have both genetic and environmental components. PPMs could be a proxy measure of physical activity but there is only modest correlation between physical activity and physical fitness [179].

Whether the effect of childhood physical fitness persists into adult life is unknown. The aim of this prospective cohort study was, therefore, to estimate the associations between PPMs including physical work capacity at 170 heart beats per minute (PWC_{170}), time taken in the long-run and short-run, number of sit-ups completed, and leg muscle strength in childhood, and adult knee joint tibial cartilage volume and tibial bone area 25 years later.

4.2 Materials and Methods

4.2.1 Study population

Participants were selected from the Childhood Determinants of Adult Health (CDAH) Knee Study. It was a follow-up study on a sub-sample (n=330, mean age 36, range 31-42) of participants (n=2410, mean age 31) in the CDAH study during the period of 2004 to 2006. The CDAH study was a 20-year follow-up of children who participated in the Australian Schools Health and Fitness Survey (ASHFS) conducted in 1985 [162]. Detailed descriptions of each of these studies are described in section 3.5. 330 participants underwent MRI scans of their dominant knee.

4.2.2 Anthropometric measurements

Weight was measured to the nearest 0.5 kg in 1985 and 0.1 kg at follow-up with shoes, socks, and bulky clothing removed. Height was measured to the nearest 0.1 cm (with shoes and socks removed) using a stadiometer. Body mass index (BMI) was calculated as kilograms of weight per square metre of height at both time points.

4.2.3 Childhood physical performance measures

PPMs including a 1.6-km long-run, 50-m short-run and number of sit-ups were performed in childhood only. Leg muscle strength and PWC_{170} were measured at childhood in a subset of children aged 9, 12, or 15 years and these measures were repeated in their adulthood after 20 years in CDAH study. Detailed descriptions of each of these measurements are described in section 3.5.

Running fitness was evaluated by a 1.6 km long-run and a 50 m short-run. Timekeepers used stopwatches to record subjects' times in minutes and seconds as they passed the finish line.

Leg muscle strength was measured to the nearest 1.0 kg using dynamometer in childhood and adulthood (TTM Muscle Meter, Tokyo, Japan). Subjects were asked to stand on the leg dynamometer with a straight back, leaning on the wall holding the handle of the dynamometer with an overhand grip. Then the subject was asked to flex their knees until an angle of 115° was obtained, at which the bar was attached to the dynamometer by a chain by the instructor. Subjects kept a firm grip on the bar and pulled upward using only their legs, and keeping the back and neck straight, as far as possible. Subjects were

instructed in each technique before testing and verbal encouragement was given until a maximal contraction was achieved. Two trials were recorded, with the mean score taken as the criterion value for leg strength. This test examines isometric strength of the whole legs, but predominantly of the quadriceps and hip extensors. The high correlation of 0.78 between this test and a specific test of quadriceps function was reported [162, 164].

Sit-ups were performed at a cadence of 20 per minute to a maximum of 100. Knees were bent to 140° and heels were kept on the ground and the subject was asked to curl-up the trunk and move the fingers up the thigh to the level of patella in time to a cadence sound tape.

Cardiorespiratory fitness was estimated based on PWC_{170}, which was assessed using a Monark bicycle ergometer [160]. Subjects were asked to cycle at a constant 60 rpm for 3 min each at three successively increasing but submaximal workloads. Heart rate was recorded at 1-min intervals at each workload using a stethoscope in childhood and an electronic heart rate monitor in adulthood. PWC_{170} was assessed by linear regression with extrapolation of the line of best fit to a heart rate of 170 beats/min. Repeatability was not assessed in our subjects but has previously been reported as an intraclass correlation coefficient (ICC) of 0.92 [161]. 261 subjects from ASHFS completed a VO_{2max} test and the correlation between PWC_{170} and VO_{2max} was found to be 0.83 [162].

Changes in PWC_{170} and leg muscle strength from childhood to adulthood were categorised into 4 categories. The subjects were categorised into high and low fitness category (above and below the mean value of PWC_{170} or leg muscle strength) in childhood and adulthood. Change in fitness category was categorised according to fitness status at both time points: above mean in childhood and adulthood, above mean in childhood and below mean in adulthood, below mean in childhood and above mean in adulthood, and below mean at both time points.

4.2.4 Knee injury status

History of knee injury or surgery was not collected in childhood ASHFS study and therefore, telephone interview in CDAH Knee Cartilage study included history of knee injury in childhood and adulthood. Injury was recorded in response to the question “Have you had a knee injury requiring non-weight bearing treatment for more than 24 hours or surgery?”

4.2.5 Knee cartilage volume

Tibial and patellar cartilage volumes were determined by means of 3D image processing on an independent work station using the software program OsiriX (Geneva, Switzerland). Detailed description of knee cartilage volume measurements are described in section 3.5.6. Total tibial cartilage volume was calculated as the sum of medial and lateral tibial cartilage volume. Femoral cartilage volume was not assessed, as we have reported that femoral cartilage volume correlate strongly with tibial cartilage volume [173].

Ten volunteers had MRI scans performed at both hospitals' MRI machines to determine the variation between machines. The CVs for cartilage volume measurements were 9.5% (lateral tibial) and 16.2% (medial tibial), and for bone area measurements were 5.9% (lateral tibial) and 5.7% (medial tibial). Bland-Altman plots were used to examine the observed difference between these machines and this difference was dependent on the magnitude of the reading. Based on these 10 volunteers, we calculated the correction equations for cartilage volume and bone area using the slope and intercept from a linear regression model where the result from one machine was regressed on the result from other machine. The corrections were performed by keeping the values of one scan location (Melbourne) as they were and then changing the values of another scan location (Sydney) by multiplying the beta (0.88, 0.94, 0.66, 1.34, respectively, for medial and lateral tibial cartilage volume, and medial and lateral tibial bone area) of linear regression and adding the constant (0.53, 0.55, 6.11, -2.97, respectively, for medial and lateral tibial cartilage volume, and medial and lateral tibial bone area).

4.2.6 Tibial bone area

The bone area of medial and lateral tibial plateau was measured manually on the three reformatted T1-weighted MR images closest to tibial cartilage in the axial plane as describe in the previous studies [7, 69, 173]. The border of the tibial articular surface was manually marked and the area was recorded. The CVs for these measures were 2.2-2.6% [7]. Total tibial bone area was calculated as the sum of medial and lateral area.

4.2.7 Cartilage defects

Knee cartilage defects were measured as previously reported [8] in an ordinal scale using the T1-weighted spoiled gradient recall MRI sagittal images and proton density-weighted fast spin echo MRI coronal images together. Grade 0 indicated a normal

cartilage, Grade 1 indicated focal blistering and low-signal intensity area in T1-weighted sagittal images with intact surface/bottom in both T1-weighted and proton density sequences. Grade 2 indicated a loss of thickness of less than 50% on surface/bottom of the cartilage. Grade 3 represented a deep ulceration with loss of thickness >50%, and Grade 4 indicated a full-thickness chondral wear with exposure of subchondral bone. A prevalent cartilage defect was defined as a cartilage defect score of ≥2 at any site within that compartment.

4.2.8 Statistical analysis

T-tests or chi-squared tests were used to assess the differences in continuous and categorical measures respectively between groups of subjects. Univariable and multivariable linear regression analyses were employed to examine the relationships of childhood PPMs with adult cartilage volume and bone area. Age at childhood, gender, duration of follow-up, BMI at childhood and adulthood, knee injury at childhood and adulthood and/or tibial bone area (for tibial cartilage volume) were examined as potential confounders. Whether or not the association of each childhood PPMs with adult cartilage volume and bone area was independent of adult fitness was assessed by further adjustment for the corresponding PPMs measured in adulthood. Interactions between sex and PPM covariates in the regressions of cartilage volume and bone area were investigated. Adjustments for multiple testing on regression results were undertaken using the Hochberg method [180]. All statistical analyses were performed on SPSS 19 for Mac (SPSS Inc., Chicago, USA).

4.3 Results

4.3.1 Characteristics of the participants

A sample of 330 subjects had MRI data in CDAH Knee Cartilage study. There were no significant differences between those who were included in this study and the total ASHFS sample in age, sex, BMI, and PPMs (Table 4.1). Only a subset (n=110) of participants had the childhood measurements of PWC_{170} and leg muscle strength. Information on the demographic and study factors of the participants in childhood and adulthood are presented in Table 4.1 and Table 4.2 respectively.

Table 4.1. Characteristics of the participants in the CDAH Knee Cartilage study and ASHFS of 1985

Study factors	Total sample (ASHFS) n=8498	CDAH Knee Study n=330	P-value
Childhood age (year)	10.9 (2.5)	10.95 (2.6)	0.712
Sex (% female)	49 (n=4164)	47 (n=155)	0.817
Childhood BMI (kg/m^2)	18.2 (2.9)	18.1 (2.6)	0.531
Long-run (minutes)	9.4 (1.9)	9.4 (1.9)	0.884
Short-run (seconds)	9.2 (1.1)	9.2 (1.1)	0.937
Sit-ups (numbers)	38 (30)	40 (31)	0.467
PWC_{170} (watts)	91.9 (38.3)[a]	95.2 (39.9)[b]	0.642
Leg muscle strength (kg)	105.0 (86.7)[a]	98.5 (56.7)[b]	0.298

Results are means (standard deviations) or percentages
P values are from t-tests of differences in means for continuous variables and chi-squared tests of differences of proportions for categorical variables.
Measurements made in sub-samples of [a] n=2650, [b] n=110
BMI: body mass index; PWC_{170}: physical work capacity at 170 beats/min;
CDAH: Childhood Determinants of Adult Health;
ASHFS: Australian Schools Health and Fitness Survey

Table 4.2. Characteristics of the adult participants in CDAH Knee Cartilage study

Variables	Values (n=330)
Adulthood age (year)	36.3 (2.8)
Adulthood BMI (kg/m^2)	25.9 (3.8)
Childhood knee injury %	8 (n=26)
Adulthood knee injury %	17 (n=56)
PWC_{170} (watts)	173.7 (51.0)[a]
Leg muscle strength (kg)	131.2 (47.6)[a]
Medial tibial cartilage volume (cm^3)	1.8 (0.6)
Lateral tibial cartilage volume (cm^3)	2.1 (0.7)
Medial tibial bone area (cm^2)	18.4 (2.6)
Lateral tibial bone area (cm^2)	13.6 (2.8)

Results are means (standard deviations) except for percentages
[a] n=110. BMI: body mass index;
PWC_{170}: physical work capacity at 170 beats/min

4.3.2 Childhood PPMs and adult tibial bone area

Childhood PPMs including PWC_{170} (Figure 4.1), leg muscle strength and number of sit-ups were significantly and positively associated with medial, and total tibial bone area in univariable and multivariable analysis (all $p<0.05$) (Table 4.3). With a shorter run time indicating greater endurance fitness (long-run) or muscular power (short-run), they were negatively associated with medial and total tibial bone area. Similarly, leg muscle strength was positively associated with tibial bone area in medial and total sites. Current PPMs such as PWC_{170} and leg muscle strength were associated with tibial bone area in young adults (data not shown); therefore, adulthood PWC_{170} and adulthood leg muscle strength were used for further adjustment for the associations between childhood PPMs (PWC_{170} and leg muscle strength) and adulthood tibial bone area. This resulted in decreases in magnitude of these associations and became of borderline significance for total tibial bone area ($P=0.052$ for PWC_{170} and $P=0.062$ for leg muscle strength) but remained significant for medial tibial bone area ($P=0.023$ for PWC_{170} and $P=0.021$ for leg muscle strength).

Table 4.3. Associations between childhood physical performance measures and adult tibial bone area

	Univariable		Multivariable*	
Bone area (cm^2)	β (95% CI)	P-value	β (95% CI)	P-value
PWC_{170} (per 10 watts)				
Medial Tibial	**0.26 (0.14,0.39)**	**<0.001**	**0.21 (0.07,0.35)**	**0.004**
Lateral Tibial	**0.29 (0.17,0.41)**	**<0.001**	**0.17 (0.02,0.31)**	**0.028**
Total Tibial	**0.55 (0.34,0.77)**	**<0.001**	**0.38 (0.15,0.60)**	**0.001**
Long run (per 10 min)				
Medial Tibial	**-6.08 (-7.54,-4.62)**	**<0.001**	**-2.08 (-3.61,-0.55)**	**0.008**
Lateral Tibial	**-6.29 (-7.84,-4.74)**	**<0.001**	-1.25 (-2.92,0.41)	0.140
Total Tibial	**-12.37 (-15.03,-9.72)**	**<0.001**	**-3.33 (-5.86,-0.81)**	**0.010**
Short run (per 10 sec)				
Medial Tibial	**-6.28 (-8.95,-3.60)**	**<0.001**	**-4.25 (-6.96,-1.54)**	**0.002**
Lateral Tibial	**-6.67 (-9.51,-3.83)**	**<0.001**	-1.77 (-4.76,1.21)	0.243
Total Tibial	**-12.95 (-17.91,-7.99)**	**<0.001**	**-6.02 (-10.54,-1.50)**	**0.009**
Sit-ups (per 10 repetitions)				
Medial Tibial	**0.21 (0.12,0.30)**	**<0.001**	**0.11 (0.03,0.19)**	**0.009**
Lateral Tibial	**0.25 (0.15,0.34)**	**<0.001**	**0.11 (0.03,0.20)**	**0.012**
Total Tibial	**0.45 (0.29,0.62)**	**<0.001**	**0.22 (0.09,0.35)**	**0.001**
Leg muscle strength (per 10 Kg)				
Medial Tibial	**0.19 (0.10,0.27)**	**<0.001**	**0.14 (0.03,0.25)**	**0.013**
Lateral Tibial	**0.17 (0.08,0.26)**	**<0.001**	0.06 (-0.06,0.18)	0.330
Total Tibial	**0.36 (0.20,0.51)**	**<0.001**	**0.20 (0.02,0.38)**	**0.033**

Dependent variable: tibial bone area. * Adjusted for sex, childhood age, childhood body mass index, childhood injury, duration of follow-up, adult body mass index, adult knee injury. Bold denotes statistically significant results

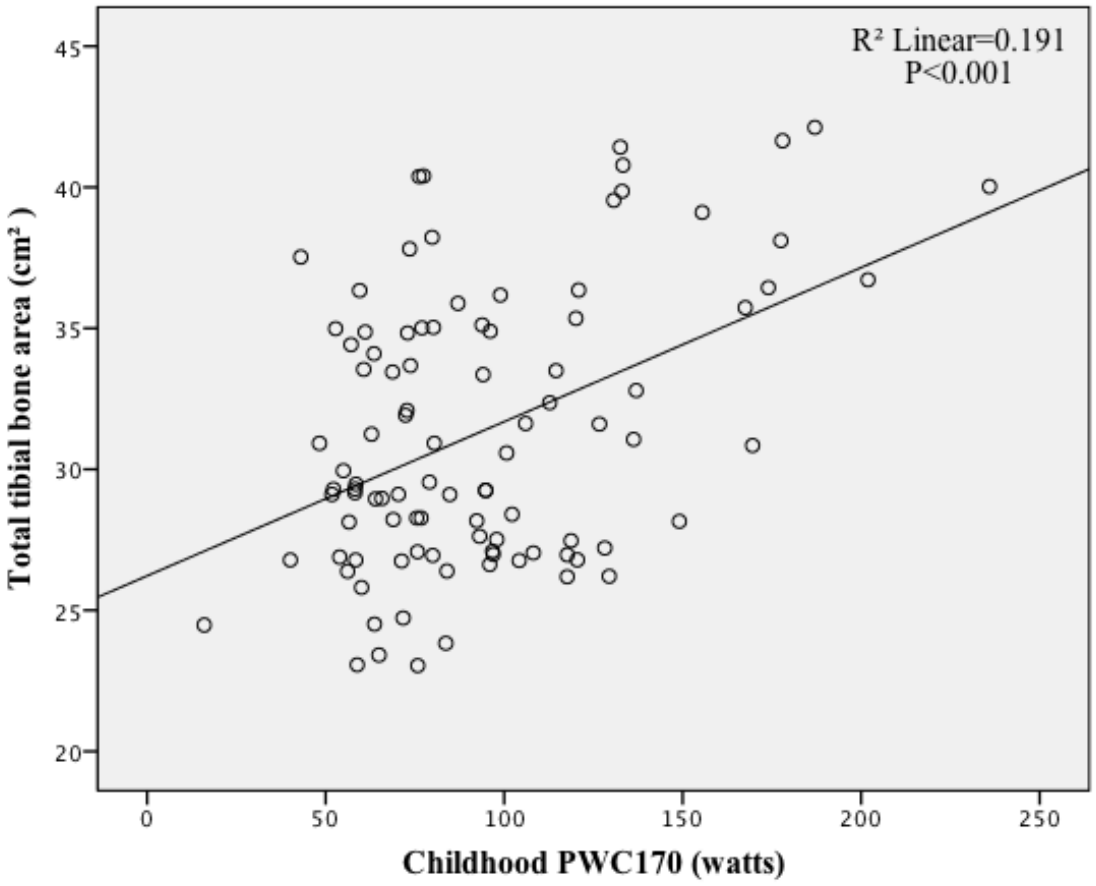


Figure 4.1. Association between childhood physical work capacity at 170 beats per minute (PWC_{170}) and total tibial bone area in adulthood

4.3.3 Childhood PPMs and adult tibial cartilage volume

All childhood PPMs including PWC_{170} (Figure 4.2), leg strength, number of sit-ups, and long-run and short-run were significantly associated with adult knee tibial cartilage volume in univariable analyses (Table 4.4, column 1). After adjustment for age at childhood, gender, duration of follow-up, BMI at childhood and adulthood, knee injury at childhood and adulthood, PWC_{170} in childhood was significantly associated with tibial cartilage volume at all sites (Table 4.4, column 2). Current PWC_{170} was associated with cartilage volume in young adults (data not shown). After adjustment for current PWC_{170}, the associations of childhood PWC_{170} with adulthood cartilage volume remained largely unchanged (medial tibial β: 0.06, 95% CI: 0.02, 0.11; lateral tibial β: 0.05, 95% CI: 0.00, 0.10; total tibial β: 0.11, 95% CI: 0.04, 0.18). The associations for medial and total tibial cartilage volume remained significant after adjustment for multiple comparisons (data not shown). The regression coefficients of the associations with medial and total tibial cartilage volume decreased by 27% to 33% but remained significant after further adjustment for tibial bone area at corresponding site (Table 4.4, column 3). Similarly, the number of sit-ups in childhood was significantly associated with medial and total tibial cartilage volume in multivariable analyses. However, these significant associations disappeared after further adjustment for tibial bone area. No significant associations of long-run, short-run, or leg muscle strength and cartilage volume were found in multivariable analyses.

Changes in PWC_{170} and leg muscle strength from childhood to adulthood were categorised into 4 categories. The subjects in high fitness category (above mean of PWC_{170}) in childhood and adulthood had a trend towards higher total cartilage volume compared to subjects who were in low fitness category at both time points in multivariable analysis (β: 0.67, 95% CI: -0.02, 1.36). Participants in high leg muscle strength category (above mean of leg muscle strength) in childhood and adulthood was not associated with higher cartilage volume compared to subjects who were in low leg muscle strength category at both time points in multivariable analysis (β: 1.00, 95% CI: -0.37, 2.36).

Out of the childhood PPMs, only PWC_{170} was associated with patellar cartilage volume in multivariable analysis (β=0.05, p=0.033). Prevalence of knee cartilage defects in this cohort was 6.7%. There were no significant associations of physical performance measures in childhood with adulthood tibial cartilage defects and adjusting for cartilage defects didn't make any changes to our results (data not shown).

Table 4.4. Association between childhood physical performance measures and adult knee cartilage volume

Cartilage Volume (cm^3)	Univariable β (95% CI)	P-value	Multivariable* β (95% CI)	P-value	Multivariable** β (95% CI)	P-value
PWC_{170} (per 10 watts)						
Medial Tibial	**0.05 (0.03,0.08)**	**<0.001**	**0.06 (0.02,0.10)**	**0.006**	**0.04 (0.00,0.08)**	**0.044**
Lateral Tibial	**0.07 (0.04,0.10)**	**<0.001**	**0.05 (0.00,0.09)**	**0.036**	0.04 (-0.01,0.08)	0.100
Total Tibial	**0.12 (0.08,0.17)**	**<0.001**	**0.11 (0.05,0.17)**	**0.001**	**0.08 (0.02,0.14)**	**0.010**
Long run (per 10 min)						
Medial Tibial	**-0.67 (-1.01,-0.32)**	**<0.001**	-0.08 (-0.52,0.36)	0.714	0.11 (-0.32,0.53)	0.613
Lateral Tibial	**-1.10 (-1.50,-0.70)**	**<0.001**	-0.14 (-0.62,0.35)	0.582	-0.05 (-0.53,0.42)	0.824
Total Tibial	**-1.76 (-2.35,-1.18)**	**<0.001**	-0.22 (-0.87,0.44)	0.513	0.09 (-0.53,0.71)	0.777
Short run (per 10 sec)						
Medial Tibial	**-0.68 (-1.28,-0.07)**	**0.028**	-0.07 (-0.85,0.72)	0.869	0.33 (-0.43,1.09)	0.396
Lateral Tibial	**-1.23 (-1.94,-0.52)**	**0.001**	-0.58 (-1.44,0.28)	0.188	-0.46 (-1.31,0.38)	0.283
Total Tibial	**-1.91 (-2.95,-0.87)**	**<0.001**	-0.65 (-1.81,0.52)	0.277	-0.09 (-1.19,1.02)	0.880
Sit-ups (per 10 repetitions)						
Medial Tibial	**0.04 (0.02,0.06)**	**<0.001**	**0.03 (0.01,0.05)**	**0.013**	0.02 (0.00,0.04)	0.078
Lateral Tibial	**0.03 (0.01,0.06)**	**0.006**	0.01 (-0.02,0.03)	0.466	0.00 (-0.02,0.03)	0.872
Total Tibial	**0.07 (0.04,0.11)**	**<0.001**	**0.04 (0.00,0.07)**	**0.026**	0.02 (-0.01,0.05)	0.249
Leg muscle strength (per 10 Kg)						
Medial Tibial	**0.02 (0.00,0.04)**	**0.020**	0.01 (-0.03,0.04)	0.679	-0.01 (-0.04,0.03)	0.698
Lateral Tibial	**0.04 (0.01,0.06)**	**0.002**	0.01 (-0.03,0.05)	0.591	0.01 (-0.03,0.04)	0.752
Total Tibial	**0.06 (0.03,0.09)**	**0.001**	0.02 (-0.03,0.07)	0.507	0.00 (-0.05,0.05)	0.947

Dependent variable: tibial cartilage volume. * Adjusted for sex, childhood age, childhood body mass index, childhood injury, duration of follow-up, adult body mass index, adult knee injury; ** further adjustment for tibial bone area.
Bold denotes statistically significant results.

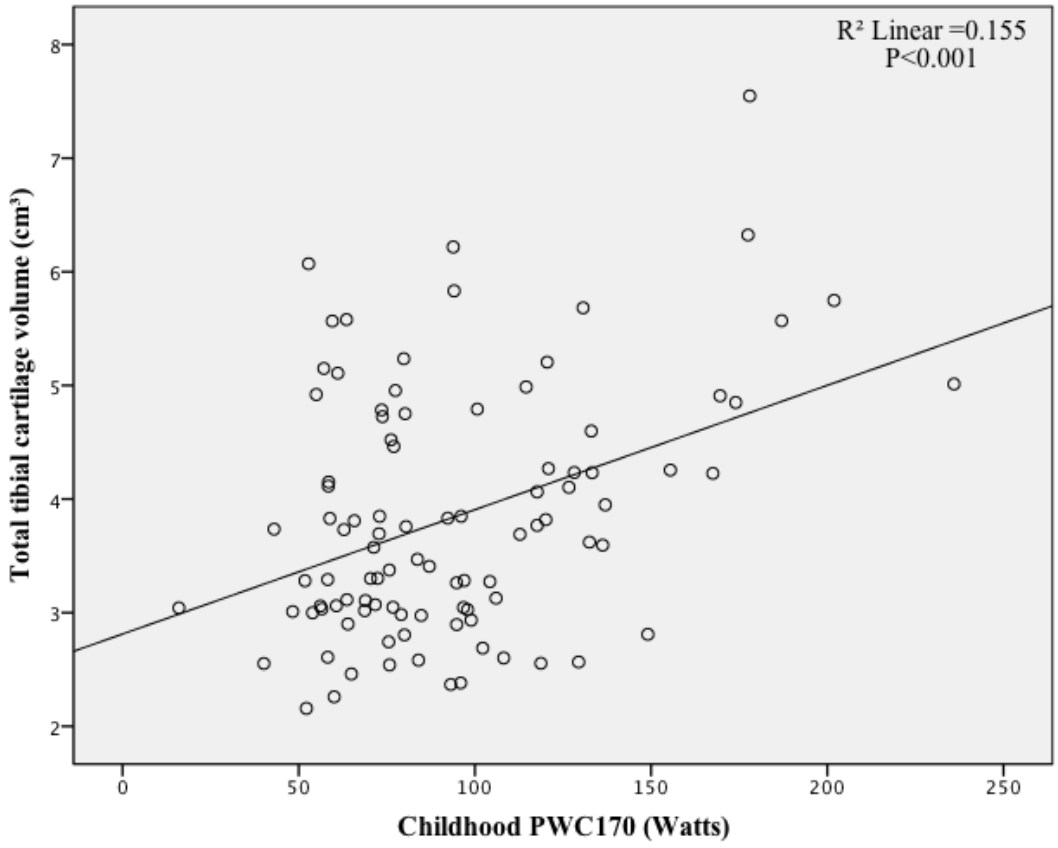


Figure 4.2. Association between childhood physical work capacity at 170 beats per minute (PWC_{170}) and total tibial cartilage volume in adulthood

4.4 Discussion

To our knowledge, this is the first long-term investigation of the associations between childhood PPMs and knee structures among adults. We found that childhood PPMs were consistently and positively associated with adult knee tibial bone area 25 years later. Higher childhood PWC_{170} and sit-ups were associated with greater adult tibial cartilage volume in multivariable analyses, and these associations decreased in magnitude but remained statistically significant for PWC_{170} after adjustment for tibial bone area, suggesting they are, at least in part, mediated by higher bone area. Lastly, the associations between childhood PPMs and adult cartilage volume remained after adjustment for the corresponding available adult fitness measure, suggesting childhood fitness affects adult knee structures independently of adult attained fitness.

Tibial bone area and cartilage volume are common measurable indices of knee joint health. Tibial bone area is an approximation of the cross-sectional surface area on the top of the tibial bone that is covered by tibial cartilage. This forms the site for the dispersion of mechanical loading distributed by the femur, and is considered a dynamic structure that can respond to physical stimuli. Among older adults, tibial bone area is positively associated with cartilage defects and cartilage volume loss [17] and has been shown to be an independent predictor of knee replacement over 4 years [19]. The tibial bone area increase in older populations appears maladaptive and is possibly due to remodelling of subchondral trabeculae with increased extracellular matrix deposition as a result of excessive load on the bone [17]. This may not be generalised to all age groups, because increased bone area during growth may reflect adaptive change. Hypothetically, a greater tibial plateau will limit stresses in the joints because higher loads are distributed over a greater surface [23]. Evidence suggests that functional adaptations can occur in response to mechanical stimuli in the subchondral bone [181]. There is, as yet, no evidence to suggest whether increased tibial bone area among young adults is associated with OA in later life. We found that all childhood PPMs including PWC_{170} were positively associated with tibial bone area in medial and total sites. Our data would favour adaptive change, because tibial bone area was associated with a larger cartilage volume. This is consistent with the results of a cross-sectional study of athletes in which bone size was found to be larger than controls [20]. Therefore, a higher bone area in young adults could be protective and may help the development of larger cartilage volume.

Cartilage volume is the amount of cartilage that covers the top of tibia, patella and femur and was measured in this study using MRI techniques. Increased cartilage volume is

associated with reduced radiographic OA [7], reduced cartilage defects [8], reduced knee symptoms [9] and predicts decreased knee joint replacement over time [182]. Loss of cartilage volume appears to start after the age of 40 although there is a continuum in cartilage loss [183]. Subjects in our study were of mean age 36 and are therefore expected to have a maximum cartilage volume in their lifetime because cartilage volume loss mainly starts after 40 years. Therefore, it is likely that a higher cartilage volume, similar as the peak bone mass that is a strong predictor of future risk of osteoporosis in older people [10], should be protective against the development of OA in later life. This statement is speculative and needs further studies to prove this concept. We found that childhood PWC_{170} was positively associated with tibial cartilage volume at the medial and total sites. These associations were independent of adult PWC_{170} indicating that along with genetic factors, there is a great influence of environmental factors in the association of childhood fitness on adult cartilage volume. Similar associations were noted in adult population (mean age 45 years), for which change in PWC_{170} was positively associated with change in medial and lateral tibial cartilage volume and baseline PWC_{170} was positively associated with change in tibial bone area at lateral and total sites [75].

In addition, other childhood PPMs including the number of sit-ups were associated with increased tibial cartilage volume among adults. The associations between sit-ups and cartilage volume became non-significant after further adjustment for tibial bone area suggesting a higher cartilage volume gain with sit-ups was through the development of a greater bone area. While long-run, short-run and leg muscle strength were significantly associated with adult tibial bone area, they were not associated with adult tibial cartilage volume. The reasons behind these varying associations are unknown but could be due to the low correlation (-0.33) of long-run with VO_2 max measurement as opposed to PWC_{170} which had a high correlation of 0.83. In previous studies, lower limb muscle strength was not associated with articular cartilage accrual over 2 years in children aged between 9 to 18 years, [149] but lower limb muscle strength at baseline was positively associated with change of total tibial bone area and tibial/femoral cartilage volume over 2 years in middle-aged adults [75, 98]. When we further adjusted tibial cartilage volume results for tibial bone area, the associations remained statistically significant only for PWC_{170} with the size of the response being decreased by about one third, suggesting higher childhood fitness measures lead to an increase in cartilage volume partially through greater bone area. The consistent associations with PWC_{170} could be due to the more accurate measurement of fitness over the other measurements or that joint structure is more responsive to cardio-metabolic fitness than other PPMs.

There is a strong genetic contribution to bone area and cartilage volume based on the results of our previous studies, and shared genes with PPMs may explain this association [184-186]. We found that the association of childhood PWC_{170} with adulthood bone area was in part dependent of the adulthood PWC_{170} measure. However, the associations with cartilage volume and medial tibial bone area remained significant after we adjusted for the corresponding available adult fitness measure, suggesting these are likely to reflect the effect of environment including their physical activity. Childhood fitness adjusted for adulthood fitness technically reflects the change in fitness. Change in fitness is likely to be a good proxy for change in physical activity, as supported by the finding that physical activity measured by pedometer in adulthood was higher in the persistent-high fitness or increasing-fitness groups [187].

Our results were consistent with the findings for the association between childhood fitness and adult bone mass. In the CDAH study, childhood PWC_{170}, long-run and short-run were significantly associated with increased heel bone mass assessed using a bone ultrasound densitometer, whereas there were no significant associations with leg muscle strength [162]. In this context, we can speculate that tibial bone accrual from childhood to early adulthood is a physiological rather than pathological process, and tibial bone growth under effects of PPMs is beneficial for the development of articular cartilage. Future studies in other cohorts are desirable to confirm this.

A strength of our study was the use of 25-year prospective data with a large number of objectively-assessed PPMs in childhood and adulthood. A potential limitation is that the response rate was low with only 43% of the persons invited to participate having MRI performed. They represented <5% of the original participants in ASHFS. Reassuringly, there were no significant differences in age, sex, BMI, PPMs and knee injury between those with and without MRI scans, or between subjects included in this study and the remainder of the original cohort (ASHFS), or between subjects with fitness measures and the remainder of the CDAH Knee Cartilage study participants, which suggests there was not major selection bias introduced. We did not have MRI measurements in childhood, but we have previously reported positive associations between physical activity and tibial cartilage volume among children [149]. We utilised tibial bone area and cartilage volume measurements as the joint health indicators; however, there are no studies showing that higher knee cartilage volume/bone area in young adulthood predicts less OA in later life so far so their clinical relevance is largely unknown and needs to be demonstrated by long-term longitudinal studies. We have a limited number of subjects (n=10) for the correction

of bias between the two different machines due to the difficulty in getting patients between the scanners.

In conclusion, childhood PPMs were significantly associated with increased knee tibial bone area and childhood PWC_{170} was associated with increased cartilage volume in adulthood. The associations between fitness measures and tibial cartilage volume appear to be mediated in part by tibial bone area. This suggests physical performance measures in childhood can independently influence adult knee joint structure possibly through adaptive mechanisms during growth.

Chapter 5 - The association between childhood overweight measures and adulthood knee pain, stiffness and dysfunction: a 25-year cohort study

5.1 Introduction

Obesity is a major public health problem among adults and, more recently, among children around the world. The rise in obesity has contributed to the epidemic of non-communicable diseases such as type-2 diabetes, hypertension and coronary heart diseases. Obesity has also been strongly associated with musculoskeletal conditions especially osteoarthritis (OA) [108].

The major importance of childhood overweight arises from the fact that it predicts adult obesity [105]. Adolescents who were overweight were almost 18 times more likely than their leaner peers to be obese in early adulthood [106]. We have also found that obesity in childhood was strongly predictive of obesity in early adulthood [107]. However, childhood obesity also affects childhood health. There is evidence for a higher prevalence of lower extremity malalignment, fractures and musculoskeletal pain among obese children than among normal weight children [111].

The knee joint is commonly affected by pain in both overweight paediatric [111] and adult populations [123]. Up to half of people aged 50 years or over reported having knee pain during one year, and a quarter reported having severe and disabling knee pain [119]. Studies in adults have established a link between obesity and the subsequent development or progression of knee pain [119, 124]. Most of these studies were conducted in older populations, and only one study has reported a deleterious association of childhood weight with adulthood knee pain and in this study the classification of childhood obesity was based on adulthood BMI scales and a validated knee pain questionnaire was not used [127]. Therefore, we hypothesised that childhood overweight measures were associated with increased adulthood knee pain. The aim of our study was, therefore, to describe the associations between overweight measures in childhood and knee pain, stiffness and dysfunction among adults 25 years later.

5.2 Materials and Methods

5.2.1 Study population

The CDAH Knee Cartilage study a follow-up study on a sub-sample (n=449, aged 31-41 years, female 47.9%) of participants who completed the Childhood Determinants of Adult Health (CDAH) study during 2004 to 2006. The CDAH study was a 20-year follow-up of children (n=2410, mean age 31) who participated in the Australian Schools Health and Fitness Survey (ASHFS) conducted in 1985. Detailed descriptions of each of

these studies are given in section 3.5. 449 subjects were asked to complete a computer assisted telephone interview (CATI), which included knee pain, stiffness and dysfunction questionnaires.

5.2.2 Anthropometric measurements

Weight was measured to the nearest 0.5 kg in 1985 and 0.1 kg at follow-up with shoes, socks, and bulky clothing removed. Height was measured to the nearest 0.1 cm (with shoes and socks removed) using a stadiometer. Body mass index (BMI) was calculated as kilograms of weight per square meter of height for both time points. Overweight in childhood was defined according to age and sex specific cutpoints [158]. Adulthood overweight was defined as a BMI above 25 kg/m^2. Change in overweight status was categorised according to overweight status at both time points: normal in childhood and adulthood, normal in childhood and overweight in adulthood, overweight in childhood and normal in adulthood, and overweight at both time points.

5.2.3 Knee symptom measurements

Information on knee pain and injury were collected during the time of CATI. We used the Western Ontario MacMaster Universities (WOMAC) osteoarthritis index for assessing knee pain, stiffness and physical dysfunction during the past 30 days on a scale of 0 to 9 where 0 indicated no complaints and 9 indicated the maximum intensity of the complaint. WOMAC scale is an established scale for OA research and is also validated for responsiveness of knee complaints in young population without OA [169]. WOMAC knee pain was reported in five subscales: pain experienced when walking on level surface, going up and/or down stairs, sitting/lying, rest at night and standing upright. WOMAC stiffness and dysfunction were reported in two and seventeen subscales, respectively. Total WOMAC scores were calculated by adding the scores of each subscale. Presence of any pain, stiffness and dysfunction was defined as any score >0. History of knee injury during childhood and adulthood were also recorded separately during CATI in response to the question “Have you had a knee injury requiring non-weight bearing treatment for more than 24 hours or surgery?”

5.2.4 Statistical analysis

Our primary objective was to describe the association of childhood overweight with adulthood total WOMAC knee pain scale. We performed further exploratory analyses with the WOMAC knee pain subscale scores particularly walking knee pain since it was the most biologically plausible subscale associated with obesity. We also analysed the associations between childhood overweight and adulthood stiffness and dysfunction. These associations were tested using childhood weight and BMI as continuous measures and overweight (Yes/No) as a categorical variable. Unpaired T-tests or chi-squared tests were used to assess the difference between groups based on the presence or absence of knee pain when walking on level surface. Univariable and multivariable log binomial regression was used to estimate relative risk (RR) for associations between childhood overweight measures and adult knee pain, stiffness, and dysfunction before and after adjustment for potential confounders. If the log binomial model failed to converge, RR was estimated using a Poisson distribution and robust standard errors [188]. Age at childhood, duration of follow-up, height (if weight was the predictor), smoking status, socioeconomic status (based on the category of occupation for the longest period) and knee injury at childhood and adulthood were examined as potential confounders based on the significant associations with childhood overweight measures or knee pain and from our previous findings of these being important covariates. The independent associations of each childhood overweight measures were assessed by further adjustment for the corresponding adulthood obesity measure. Interactions between sex and overweight measures on knee pain, stiffness or dysfunction were investigated by regressing knee pain, stiffness and dysfunction on a binary (0/1) term for sex within a covariate, and assessed by testing the statistical significance of the coefficient of a (sex × covariate score). We decided to separate men and women for analysis based on the distinct gender differences in our results. All statistical analyses were performed using SPSS 19 for Mac (SPSS Inc., Chicago, IL, USA).

5.3 Results

5.3.1 Characteristics of the participants

A sample of 449 subjects was included in this analysis. The overall prevalence of total WOMAC knee pain was 34% (range: 0-31, mean: 1.76). Prevalence of knee pain walking on a level surface was 10% and prevalence of overweight in childhood and adulthood was 7% and 48%, respectively, in this cohort. The prevalence of total WOMAC stiffness (range: 0-17, mean: 1.16) and dysfunction (range: 0-125, mean: 4.25) were 33% and 41%, respectively. The sample we derived for this study was a representative of the original cohort and there were no significant differences between these participants, the rest of total ASHFS sample and CDAH sample for age, sex, BMI, and overweight status (data not shown). Demographic and study factors of the participants based on whether they experienced any knee pain (split by gender) are presented in Table 5.1. There were no significant differences in age (childhood and adulthood), childhood knee injury, BMI and weight between those with WOMAC pain and without pain. However, the proportions of childhood overweight and adulthood injury were greater for male subjects with any knee pain.

Table 5.1. Characteristics of the participants based on whether they experienced knee pain (total WOMAC pain >0).

	No pain	**Pain**	**p-value**
Total	**(n=295)**	**(n=154)**	
Childhood Age (year)	11.2 (2.6)	11.0 (2.6)	0.468
Childhood BMI (kg/m^2)	18.2 (2.6)	18.1 (2.6)	0.631
Childhood Weight (kg)	40.8 (13.0)	40.0 (13.1)	0.517
Childhood Overweight (%)	6 (n=18)	9 (n=14)	0.127*
Adulthood BMI (kg/m^2)	25.1 (3.8)	25.5 (4.3)	0.268
Sex (female, %)	48 (n=141)	48 (n=74)	0.519*
Childhood Injury (%)	8 (n=24)	10 (n=15)	0.306*
Adulthood Injury (%)	15 (n=44)	25 (n=38)	**0.007***
Females	**(n=141)**	**(n=74)**	
Childhood Age (year)	10.9 (2.7)	11.1 (2.5)	0.660
Childhood BMI (kg/m^2)	18.2 (2.6)	18.1 (2.6)	0.658
Childhood Weight (kg)	39.4 (11.6)	39.2 (11.5)	0.891
Childhood Overweight (%)	7 (n=11)	5 (n=4)	0.447*
Adulthood BMI (kg/m^2)	24.2 (3.8)	24.8 (5.0)	0.370
Childhood Injury (%)	3 (n=4)	8 (n=6)	0.133*
Adulthood Injury (%)	11 (n=15)	16 (n=11)	0.212*
Males	**(n=154)**	**(n=80)**	
Childhood Age (year)	11.4 (2.5)	10.9 (2.7)	0.149
Childhood BMI (kg/m^2)	18.2 (2.6)	18.1 (2.6)	0.810
Childhood Weight (kg)	42.1 (14.1)	40.7 (14.5)	0.478
Childhood Overweight (%)	4 (n=7)	12 (n=10)	**0.028***
Adulthood BMI (kg/m^2)	25.8 (3.6)	26.2 (3.5)	0.437
Childhood Injury (%)	13 (n=20)	12 (n=9)	0.546*
Adulthood Injury (%)	19 (n=29)	34 (n=27)	**0.009***

Results are mean (standard deviation) except for percentages
* Chi-square tests, and t-tests for others

5.3.2 Childhood overweight measures and adult WOMAC knee pain

Analyses using total WOMAC knee pain score (yes/no) as the outcome (Table 5.2) showed no significant association between childhood overweight measures and total knee pain. However, sex specific analysis revealed an effect of being overweight in childhood on adult total WOMAC pain for males but not for females. This association persisted after further adjustment for adulthood overweight status.

When considering type of pain, childhood overweight measures including weight, BMI and being overweight (yes/no) were significantly and positively associated in multivariable analysis with knee pain when walking in adulthood after adjustment for covariates (all $p<0.05$) (Table 5.3). This association was independent of the adulthood overweight measures, and was stronger and only significant for males.

Table 5.2. The association of childhood overweight measures with total WOMAC knee pain score in adulthood.

	Univariable RR (95% CI)	**Multivariable* RR (95% CI)**	**Multivariable** RR (95% CI)**
Total			
BMI (per kg/m^2)	1.00 (0.98,1.03)	1.00 (0.94,1.06)	0.99 (0.92,1.06)
Overweight (yes vs no)	1.38 (0.92,2.07)	1.25 (0.82,1.91)	1.22 (0.79,1.88)
Weight (per kg)	1.00 (1.00,1.01)	1.00 (0.98,1.03)	1.00 (0.97,1.03)
Females			
BMI (per kg/m^2)	0.99 (0.92,1.07)	0.97 (0.89,1.06)	0.95 (0.86,1.05)
Overweight (yes vs no)	0.86 (0.37,1.99)	0.80 (0.33,1.95)	0.79 (0.32,1.95)
Weight (per kg)	1.00 (0.98,1.02)	0.99 (0.95,1.03)	0.99 (0.94,1.03)
Males			
BMI (per kg/m^2)	0.99 (0.92,1.06)	1.05 (0.96,1.14)	1.04 (0.94,1.14)
Overweight (yes vs no)	**1.82 (1.17,2.84)**	**1.72 (1.11,2.69)**	**1.68 (1.06,2.65)**
Weight (per kg)	1.00 (0.98,1.01)	1.02 (0.98,1.05)	1.02 (0.98,1.05)

* Adjusted for age, sex, height (for weight), duration of follow-up, child and adult knee injury, smoking status, socioeconomic position

** Further adjusted for adult corresponding measure

RR is Relative Risk and bold denotes statistical significance

Table 5.3. The association of childhood overweight measures with knee pain when walking on a surface in adulthood.

	Univariable	**Multivariable***	**Multivariable****
	RR (95% CI)	**RR (95% CI)**	**RR (95% CI)**
Total			
BMI (per kg/m^2)	**1.11 (1.02,1.20)**	**1.13 (1.02,1.26)**	**1.13 (1.00,1.26)**
Overweight (yes vs no)	**2.92 (1.50,5.69)**	**2.64 (1.29,5.40)**	**2.68 (1.29,5.60)**
Weight (per kg)	1.01 (0.99,1.03)	**1.04 (1.00,1.09)**	**1.04 (1.00,1.09)**
Females			
BMI (per kg/m^2)	1.09 (0.96,1.23)	1.09 (0.94,1.27)	1.03 (0.86,1.22)
Overweight (yes vs no)	1.70 (0.44,6.58)	1.38 (0.33,5.80)	1.28 (0.30,5.48)
Weight (per kg)	1.01 (0.98,1.04)	1.03 (0.96,1.10)	1.01 (0.93,1.09)
Males			
BMI (per kg/m^2)	**1.12 (1.01,1.25)**	**1.17 (1.01,1.35)**	**1.34 (1.13,1.58)**
Overweight (yes vs no)	**3.83 (1.78,8.25)**	**3.65 (1.60,8.33)**	**4.29 (1.83,10.02)**
Weight (per kg)	1.01 (0.98,1.03)	**1.06 (1.00,1.12)**	**1.08 (1.02,1.15)**

* Adjusted for age, sex, height (for weight), duration of follow-up, child and adult knee injury, smoking status, socioeconomic position
** Further adjusted for adult corresponding measure
RR is Relative Risk and bold denotes statistical significance

5.3.3 Childhood overweight measures and adult total WOMAC knee stiffness

Similar to total WOMAC knee pain, childhood BMI and weight were significantly and positively associated with the presence of adult total WOMAC knee stiffness in multivariable analysis for males (but not for females) after adjustment for covariates (all $p<0.05$) (Table 5.4). These associations remained unchanged after further adjustment for the corresponding current overweight measures.

Table 5.4. The association of childhood overweight measures with total WOMAC knee stiffness in adulthood

	Univariable RR (95% CI)	**Multivariable* RR (95% CI)**	**Multivariable** RR (95% CI)**
Total			
BMI (per kg/m^2)	1.03 (0.98,1.09)	1.06 (1.00,1.12)	1.02 (0.96,1.09)
Overweight (yes vs no)	1.01 (0.60,1.72)	0.96 (0.57,1.60)	0.88 (0.52,1.48)
Weight (per kg)	1.00 (0.99,1.01)	1.02 (1.00,1.04)	1.01 (0.99,1.04)
Females			
BMI (per kg/m^2)	0.99 (0.90,1.08)	0.98 (0.88,1.09)	0.93 (0.81,1.05)
Overweight (yes vs no)	0.55 (0.15,1.99)	0.50 (0.13,2.01)	0.47 (0.11,1.96)
Weight (per kg)	1.00 (0.98,1.02)	0.98 (0.93,1.03)	0.97 (0.92,1.02)
Males			
BMI (per kg/m^2)	**1.06 (1.01,1.12)**	**1.11 (1.05,1.19)**	**1.10 (1.02,1.18)**
Overweight (yes vs no)	1.28 (0.75,2.18)	1.20 (0.71,2.05)	1.10 (0.65,1.89)
Weight (per kg)	1.00 (0.99,1.01)	**1.04 (1.01,1.07)**	**1.03 (1.01,1.06)**

* Adjusted for age, sex, height (for weight), duration of follow-up, child and adult knee injury, smoking status, socioeconomic position
** Further adjusted for adult corresponding measure
RR is Relative Risk and bold denotes statistical significance

5.3.4 Childhood overweight measures and adult total WOMAC physical dysfunction

Childhood overweight measures were not associated with adult total WOMAC dysfunction in the sample overall and for females (Table 5.5). However, among males, all childhood overweight measures were positively associated with total knee dysfunction after adjustment for covariates. All these associations weakened but persisted after further adjustment for the corresponding adulthood overweight measures except the association of childhood weight.

Table 5.5. The association of childhood overweight measures with total WOMAC physical dysfunction in adults.

	Univariable RR (95% CI)	**Multivariable* RR (95% CI)**	**Multivariable** RR (95% CI)**
Total			
BMI (per kg/m^2)	1.02 (0.98,1.07)	1.04 (0.99,1.09)	1.02 (0.96,1.08)
Overweight (yes vs no)	**1.42 (1.02,2.00)**	1.34 (0.95,1.90)	1.29 (0.90,1.83)
Weight (per kg)	1.00 (0.99,1.01)	1.02 (0.99,1.04)	1.01 (0.99,1.03)
Females			
BMI (per kg/m^2)	0.99 (0.92,1.05)	0.98 (0.91,1.06)	0.95 (0.87,1.04)
Overweight (yes vs no)	1.11 (0.60,2.04)	1.01 (0.55,1.87)	1.00 (0.54,1.86)
Weight (per kg)	0.99 (0.98,1.01)	0.99 (0.96,1.03)	0.99 (0.95,1.02)
Males			
BMI (per kg/m^2)	1.05 (1.00,1.11)	**1.10 (1.03,1.17)**	**1.08 (1.00,1.16)**
Overweight (yes vs no)	**1.70 (1.15,2.51)**	**1.61 (1.07,2.43)**	**1.52 (0.99,2.32)**
Weight (per kg)	1.01 (0.99,1.02)	**1.03 (1.00,1.06)**	1.02 (1.00,1.05)

* Adjusted for age, sex, height (for weight), duration of follow-up, child and adult knee injury, smoking status, socioeconomic position
** Further adjusted for adult corresponding measure
RR is Relative Risk and bold denotes statistical significance

5.3.5 Change in overweight status from childhood to adulthood and walking WOMAC pain

Change in overweight status was significantly associated with adult knee pain when walking, with subjects who were overweight in both childhood and adult life (5%) having the greatest proportion and risk of knee pain (27.3% having knee pain, RR: 2·42, 95% CI: 1·06, 5·53) compared with those who had normal weight in both childhood and adult life (51%, 8.6% having knee pain) (Figure 5.1). Also, subjects who were overweight in childhood and then of normal weight in adulthood (2%) also had higher prevalence (25%) and risk (RR: 1.92, 95% CI: 0.46, 8.03) of knee pain compared to subjects who were normal in childhood and became overweight in adulthood (43%, 9.8% having knee pain). A similar trend was observed when we looked at the change in overweight with total WOMAC pain (Figure 5.2).

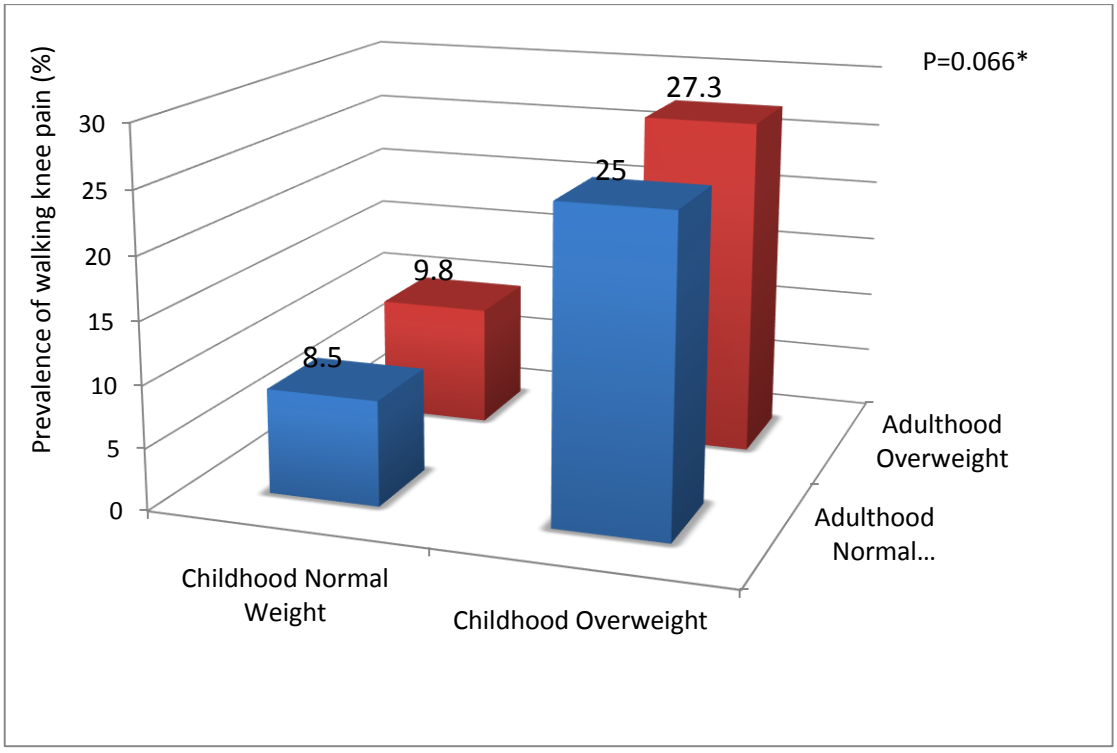


Figure 5.1. Prevalence of adult knee pain when walking for subjects classified by their change in overweight status from childhood to adulthood

(P-value is from log likelihood ratio test)

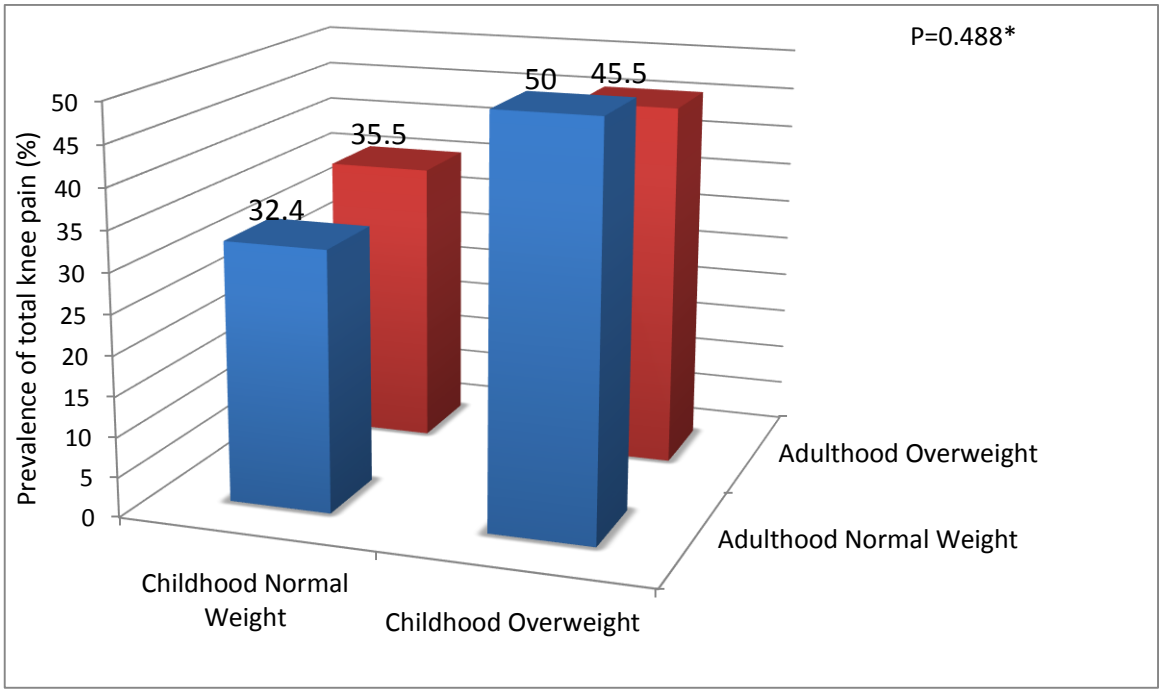


Figure 5.2. Prevalence of adult total WOMAC knee pain for subjects classified by their change in overweight status from childhood to adulthood

(P-value is from log likelihood ratio test)

5.4 Discussion

This is the first long-term cohort study, to our knowledge, to study the associations between childhood overweight measures and knee pain, stiffness and dysfunction in adults. Overall, there were no significant associations between childhood overweight measures and total WOMAC knee pain, stiffness and dysfunction scores. However, childhood overweight measures were associated with knee pain (mainly pain when walking on the flat surface), stiffness and dysfunction among males. Crucially most of the associations were shown for the continuous measure of weight and BMI as well as the categorical measures of overweight. They remained unchanged after adjustment for the corresponding adult overweight measure, suggesting childhood overweight measures can independently affect adult knee joint symptoms in males.

Our findings of childhood overweight measures predicting adult knee pain when walking at mean age of 35 is supported by a similar study that looked at the effect of BMI at 7, 11, 16, 23, 33 and 45 years on knee pain at 45 years. They found a higher risk of adulthood knee pain for the obese category relative to the underweight in each age group. Childhood BMI was associated with adult knee pain, but this association was dependent on adulthood BMI but BMI in the early 20s was an independent predictor of knee pain at 45 years [127]. This study had some limitations because adult BMI categories were used for the definition of overweight and obesity in childhood, the reference category for comparison was an underweight group rather than normal, males and females were not separated for analyses, and they did not use a validated scale for knee pain assessment.

The mechanisms underlying the associations between childhood overweight measures and adult walking knee pain are unclear. Forces transmitted through the knee joint during walking can exceed four times body weight [189]. Consequently, increases in body weight, without associated compensatory adaptations in knee joint anatomy and limb kinematics and kinetics during movement, would increase the stresses and strains in the knee joint during walking [190]. It has been reported that each pound of weight loss will result in a 4-fold reduction in the load exerted on the knee per step during daily activities, and the authors of that study suggested that a load reduction of this magnitude appeared to be clinically meaningful if accumulated over thousands of steps per day [191]. Therefore, the effects of childhood overweight on adult knee pain are most likely due to increase in joint loading and alterations in gait mechanics [192, 193].

Some prospective studies in older populations have reported similar associations between BMI and total WOMAC pain as we found for males. Jinks et al reported that

among responders with no knee pain at baseline, being obese rather than of normal weight predicted onset of severe knee pain over three years [194]. Obesity also was a strong predictor of progression of knee pain, and reduction in BMI category from obese to overweight avoided 19% of new cases of severe knee pain over a three year period [124], including almost half of the new cases that arose in the obese group [194]. In a retrospective study exploring the effects of actual weight loss in women, a loss of approximately 5.1 kg decreased the odds of developing symptomatic knee OA by 50% over 12 years [195]. Similarly, cross-sectional studies in children and adolescents of mean age 12.3 years and 17 years found a significant increase in the odds of knee pain with every unit increase in BMI [128, 196].

The male-female differences in results were consistent in this study but unexpected. The adult data suggest body fat is similarly related to pain for both males and females suggesting this association may be specific to childhood. The reasons for these sex discrepancies are unknown. The prevalence of OA in most joints is higher among men than among women before 50 years of age, but higher among women than among men after 50 years of age [56, 197]. Consistently, the incidence of symptomatic knee OA has been found to be higher among males for all age groups preceding 50 years of age [197]. This is important in context of our sample with a mean age of 35 years, in which the significant results were found only among males. In a related study we also found that BMI as a child was related to bone mass in later life among males but not females [162] and women, who were overweight in childhood, had 5% denser trabecular density with no difference in cortical density. In contrast, trabecular density in men who were overweight in childhood was not different but their cortical density was 1% lower [198], suggesting the effect of overweight in childhood is modified by sex.

The prevalence of overweight in childhood was very low as expected from a study conducted in 1985, but overweight in adulthood was much more common. Change in overweight status from childhood to adulthood was associated with increased risks of type 2 diabetes and hypertension in adults [199] and change in weight was associated with change in knee pain in adults; therefore, we hypothesised that change in overweight status would be associated with knee pain in adults. Indeed, we found that subjects who were overweight in both childhood and adult life had greater prevalence of adult knee pain when walking (27.3% of these subjects) than those of normal weight in both childhood and adult life (8.6% of these subjects). It was interesting to note that the subjects who were overweight in childhood and then of normal weight in adulthood had substantially greater prevalence of knee pain (25% of these subjects) than did subjects

who were normal in childhood and then became overweight in adulthood (9.8% of these subjects). This suggests that childhood overweight may be at least as important as adult overweight. However these results needs to be interpreted cautiously as only 7% of the childhood cohort was overweight and very few subjects (n=8) only changed their weight status from overweight in childhood to normal weight in adulthood. The underlying mechanism for why risk is maintained when changing from overweight in childhood to normal weight in adults is unknown this time.

The strength of our study was the use of 25-year prospective data with detailed questionnaires on knee pain (WOMAC scale). A potential limitation of this study is that the response rate in this sub-study was moderate (69% and 59% included in the study). Reassuringly, there were no significant difference in age, sex, and BMI between those with and without knee pain measurements in adulthood and between subjects included in this study and the remainder of the ASHFS cohort, which reduces the possibility of selection bias. The prevalence of pain in this cohort was somewhat higher than in other population cohorts. This may be due to the use of an elaborate questionnaire like WOMAC, which is more sensitive than the general questions of knee pain used in other studies and we defined knee pain, stiffness and dysfunction using any WOMAC score of >0. We did not measure malalignment in this study and therefore could not comment on the mediation of malalignment on the association between overweight measures and knee pain. We utilised weight as a predictor in this study but weight itself may not represent overweight status in childhood. However, we adjusted for height to get the best estimate of fatness. Other studies used weight as an overweight measure in childhood and reported that it was associated with increased risks for later adult-onset cancers [200]. A combination of being overweight in childhood and overweight in adulthood may simply be identifying a more severely obese population of adults; however, we also found that the continuous measures of BMI and weight in childhood were significantly associated with adult knee pain when walking, stiffness and dysfunction in males, suggesting the associations with childhood overweight would not be overestimated.

In conclusion, childhood overweight measures were significantly associated with adulthood knee mechanical joint pain, stiffness and physical dysfunction among males, independent of the adult overweight measures. Similarly, the change in overweight status from childhood to adulthood was also associated with knee pain, with subjects who were overweight in both childhood and adult life having the greatest prevalence and risk of knee pain. These data indicate that childhood obesity may lead to later adulthood knee symptoms especially in men.

Chapter 6 - Body composition, hormonal and inflammatory factors are associated with tibial cartilage volume in young adults and explain sex difference in cartilage volume

6.1 Introduction

Osteoarthritis (OA) is the most common joint disorder and the most common cause of disability in adults around the world, characterised by changes in whole joint structure including a decrease in cartilage volume. Magnetic resonance imaging (MRI) has greatly improved research in OA because of the clear visualisation of whole joint structures and the ability to manipulate the MR images to generate quantitative data on cartilage such as cartilage volume [6].

Knee cartilage volume has been used as a sensitive, accurate and reproducible outcome measure for knee OA and it is greater in males than females [150, 172], and in lateral compartment than medial compartment [150]. Given the fact that knee OA is 1.5 times more common in women than in men [61] and 4 times more common in the medial compared with the lateral compartment, low 'peak' cartilage volume in young adults may be a risk factor for knee OA in later life [62].

The associations of body composition [73, 74, 201], inflammatory [202, 203] and hormonal factors [64] with knee cartilage volume have been explored; however, these have been done mostly in middle-aged and older populations. It is unknown if the factors that influence cartilage volume in younger adults are same as those in older adults. We reported that in older adults, lean mass was positively and fat mass was negatively associated with knee cartilage volume loss [201]. Only one study has examined the associations between body composition and cartilage volume in younger adults, but it included a wide range of age from 25-60 [74]. It reported that the skeletal muscle mass rather than fat mass was positively associated with knee cartilage volume.

Inflammation can play a role in OA [204]. We have reported that in older adults, circulating levels of IL-6 and TNF-alpha were associated with knee cartilage loss [203]. Furthermore, C-reactive protein (CRP), a systemic marker of inflammation, was found to be negatively associated with knee cartilage volume in midlife women [202]. There is no data regarding the association of inflammatory factors such as CRP and fibrinogen with knee cartilage volume in younger adults.

The role of sex hormones in knee OA is still controversial. A systematic review that examined the association between OA and aspects of the fertile and menopause periods concluded that there were no or conflicting associations between female hormones and OA [65]. There were very few studies that examined the association between sex hormones and MRI- assessed knee structures [205], and to the best of our knowledge, there are no studies that have reported this association in younger adults so far. The aims of this

study were to describe the associations between body composition, hormonal and inflammatory factors and tibial cartilage volume and to explore if these factors contributed to sex difference in tibial cartilage volume in young adults.

6.2 Materials and Methods

6.2.1 Study participants

The Childhood Determinants of Adult Health (CDAH) Knee Cartilage study was conducted during 2008-2010. It was a follow-up study on a sub-sample (n=330, mean age 35, range 31-42) of participants in the CDAH study (n=2410, mean age 31). CDAH study was conducted during the period of 2004 to 2006 and included Australia-wide subjects aged between 26 to 31 years. Detailed descriptions of each of these studies are given in section 3.5. The CDAH participants were requested to have an MRI scan as part of the CDAH Knee Cartilage study. Two MRI scans were not readable and were excluded for the current analysis.

6.2.2 Anthropometric measurements

Weight was measured to the nearest 0.1 kg in CDAH study as well as in CDAH Knee Cartilage study with shoes, socks, and bulky clothing removed. Height was measured to the nearest 0.1 cm (with shoes and socks removed) using a stadiometer. Body mass index (BMI) was calculated as kilograms of weight per square metre of height. Waist to hip ratio (WHR) was calculated by dividing waist by hip circumference measured to the nearest 0.1 cm.

Skin-fold measurements were taken during the CDAH study by technicians, who were trained in accordance with the international standards of anthropometric assessment. They used anatomical landmarks to locate and measure skin-folds at tricep, bicep, subscapular and subiliac regions to the nearest 0.1 mm, using Slim Guide Calipers (SPRI Products, Libertyville, IL). Estimate of percent body fat was derived from the sum of skin-folds according to published equations for adults [163] and lean body mass (LBM) and fat mass was calculated using weight (kg) (LBM = weight – ((fat% × weight)/100)).

6.2.3 Hormone measurements

Blood samples (32 ml) were collected from the participants after an overnight fast in CDAH study. Total testosterone concentrations in female participants not on oral contraceptives were estimated by radioimmunoassay (RIA) developed by Repromed Laboratory (Dulwich, South Australia), which is sensitive for lower levels of testosterone down to 347 pmol/L. Sex hormone binding globulin (SHBG) was measured using a non-competitive liquid-phase immunoradiometric assay (SHBG-IRMA kit, Orion Diagnostica, Espoo, Finland). For testosterone, the intra- and inter- assay coefficients of variation (CVs) were 6% and 15%, respectively. For SHBG, the inter- and intra- assay CVs were 8.6% and 15.4%, respectively [167]. Free androgen index (FAI), the active testosterone in the blood, was calculated as: testosterone (nmol/L)／ SHBG (nmol/L).

6.2.4 CRP and fibrinogen

Serum CRP was determined using an automated analyzer (Olympus AU5400) and a highly sensitive turbidimetric immunoassay kit (Olympus System CRP Latex reagent, Olympus Life and Material Science Europa GmbH, Ireland) by MedVet (Institute of Medical and Veterinary Science, Adelaide, South Australia). Plasma concentration of fibrinogen was determined by the Clauss clotting method using the STA automated coagulation analyser (STA-Fibrinogen reagent, Diagnostica Stago, Manufactured in Paris, France Distributed from Parsippany, NJ, USA).

6.2.5 Tibial cartilage volume

Knee cartilage volume was assessed as described in section 3.5.6. Briefly, tibial cartilage volumes were determined by means of 3D image processing on an independent work station using the software program OsiriX (Geneva, Switzerland). Individual plates of tibial cartilage volume (medial and lateral) were isolated by manually drawing disarticulation contours around the cartilage boundaries on a section-by-section basis. The coefficients of variation (CVs) for cartilage volume measures were 2.1-2.6% [7]. Total tibial cartilage volume was calculated as the sum of medial and lateral tibial cartilage volume. Femoral cartilage volume was not assessed, as we have reported that femoral cartilage volume correlate strongly with tibial cartilage volume [173].

6.2.6 Tibial bone area

Tibial bone area was assessed as described in section 3.5.7. Briefly, The bone area of medial and lateral tibial plateau was measured manually on the three reformatted T1-weighted MR images closest to tibial cartilage in the axial plane. The border of the tibial articular surface was manually marked and the area was recorded. The CVs for these measures were 2.2-2.6% [7]. Total tibial bone area was calculated as the sum of medial and lateral area.

6.2.7 Statistical analysis

T-tests or chi-squared tests were used to assess the differences in continuous and categorical measures respectively between groups of subjects based on gender. Linear regression analyses were employed to examine the relationships of body composition, hormonal and inflammatory factors with adult tibial cartilage volume. Age at CDAH study, gender, duration of follow-up to CDAH Knee Cartilage study, BMI at CDAH Knee Cartilage study, knee injury and tibial bone area were examined as potential confounders. Interactions between sex and predictor variables on tibial cartilage volume were examined. The mediating effect of different predictors in the association of sex with cartilage volume were explored by adding each predictor one by one to the regression models and noting the changes in beta coefficient and R^2. All statistical analyses were performed on SPSS 19 for Mac (SPSS Inc., Chicago, USA).

6.3 Results

6.3.1 Characteristics of the participants

A sample of 328 subjects were included in the analysis. Characteristics of the demographic and study factors of the participants based on gender are presented in Table 6.1. Males had higher tibial cartilage volume and BMI than females at the time of MRI. BMI, LBM, LBM percentage and WHR measured 5 years prior were higher in males compared to females. Conversely, fat mass, fat mass percentage and inflammatory markers including CRP and fibrinogen were lower in males than females. Subjects did not differ in terms of age or duration of follow-up between males and females.

Table 6.1. Baseline characteristics of the participants

	Females n=155	Males n=173	P-value
Age (years)	35.3 (2.7)	35.4 (2.6)	0.776
Duration of follow-up (years)	4.5 (1.2)	4.6 (1.2)	0.564
BMI (kg/m^2)	25.0 (4.6)	26.4 (3.8)	0.002
Tibial cartilage volume (cm^3)	3.2 (0.7)	4.5 (0.9)	<0.001
5 years prior			
BMI (kg/m^2)	24.6 (4.5)	25.9 (3.7)	0.004
Fat mass (kg)	23.0 (8.1)	20.5 (7.7)	0.004
Fat percentage	33.5 (5.5)	23.9 (5.9)	<0.001
Lean body mass (kg)	44.2 (5.7)	63.0 (6.9)	<0.001
LBM percentage	66.5 (5.5)	76.1 (5.9)	<0.001
Waist hip ratio	0.7 (0.1)	0.8 (0.1)	<0.001
SHBG*(nm/l)	50.4 (26.8)		
Testosterone*(pm/l)	1493 (480)		
CRP (mg/l)	3.7 (6.2)	2.3 (5.0)	0.032
Fibrinogen (g/l)	3.2 (0.7)	2.9 (0.7)	<0.001

* Only measured in females not taking oral contraceptives
BMI: body mass index
SHBG: sex hormone-binding globulin
CRP: c-reactive protein
LBM: lean body mass
Two-tailed t tests used for differences between means; $\chi 2$ test used for proportions (percentages). Significant differences are shown in bold.
Mean (SD) except for percentages.

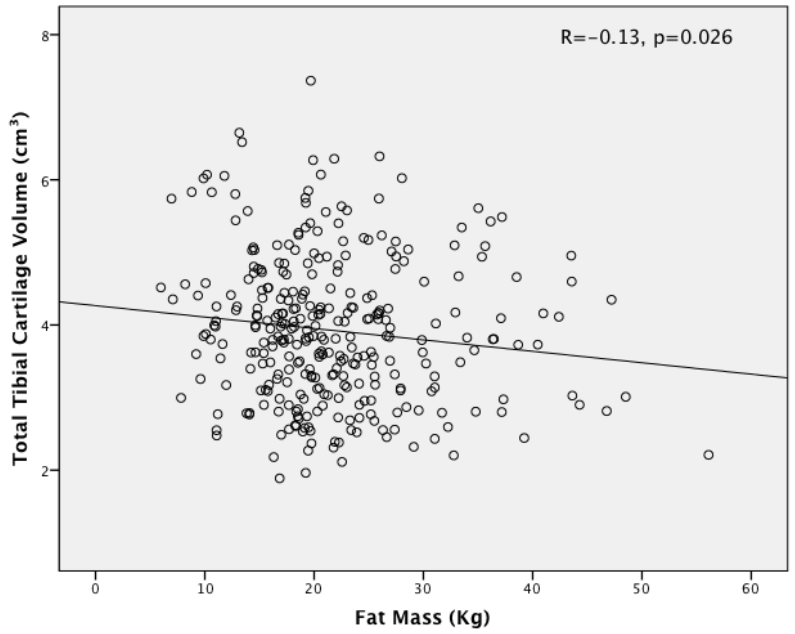
6.3.2 Obesity measures and tibial cartilage volume

BMI at the time of MRI as well as 5 years prior were not associated with total tibial cartilage volume (Table 6.2); however, fat mass (Figure 6.1a), fat mass percentage and WHR were negatively associated with tibial cartilage volume after adjustment for age, gender, duration of follow-up, injury and tibial bone size (Table 6.2). In contrast, lean body mass (Figure 6.1b) and percentage lean body mass were positively associated with tibial cartilage volume (Table 6.2). There were no significant interactions between sex with obesity measures except for WHR at p=0.1 level (p=0.062) on tibial cartilage volume.

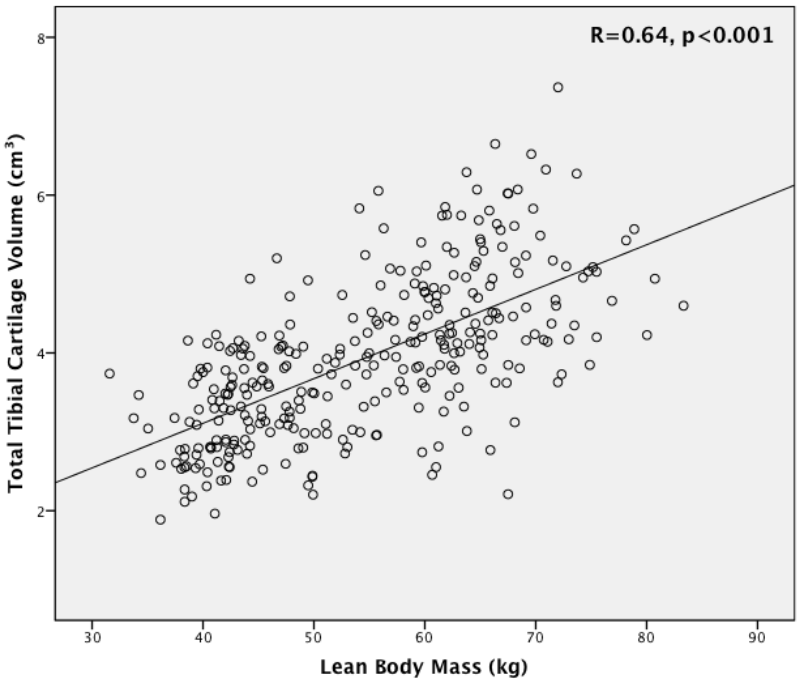
Table 6.2. Association between obesity measures assessed 5 years prior and total tibial cartilage volume

	Univariable	Multivariable*
Cartilage volume (mm^3)	β (95% CI)	β (95% CI)
Total Sample		
BMI (kg/m^2)	17.0 (-9.0,43.0)	-14.0 (-34.1,6.1)
Fat mass (kg)	**-15.7 (-29.6,-1.9)**	**-11.8 (-22.2,-1.4)**
Fat mass percentage	**-65.3 (-78.4,-52.3)**	**-22.6 (-36.7,-8.6)**
Lean body mass (kg)	**56.6 (49.1,64.1)**	**26.4 (13.6,39.1)**
LBM percentage	**65.3 (52.3,78.4)**	**22.6 (8.6,36.7)**
Waist hip ratio (per 100 unit)	**50.0 (35.9,64.0)**	**-18.5 (-36.2,-0.8)**
Females		
BMI (kg/m^2)	-10.7 (-33.6,12.2)	-20.5 (-44.4,3.3)
Fat mass (kg)	-7.0 (-20.9,6.8)	**-15.8 (-29.3,-2.3)**
Fat mass percentage	**-22.6 (-42.6,-2.6)**	**-27.9 (-46.9,-8.9)**
Lean body mass (kg)	**23.0 (3.6,42.4)**	8.2 (-13.0,29.5)
LBM percentage	**22.6 (2.6,42.6)**	**27.9 (8.9,46.9)**
Waist hip ratio	**-29.4 (-50.6,-8.3)**	**-25.5 (-46.2,-4.8)**
Males		
BMI (kg/m^2)	-5.4 (-41.7,30.8)	-7.1 (-40.5,26.4)
Fat mass (kg)	-0.1 (-17.6,17.3)	-8.4 (-24.2,7.5)
Fat mass percentage	-21.6 (-44.2,0.9)	**-18.7 (-39.4,-2.0)**
Lean body mass (kg)	**48.3 (30.5,66.2)**	**27.9 (7.3,48.5)**
LBM percentage	21.6 (-0.9,44.2)	**18.7 (2.0,39.4)**
Waist hip ratio	-9.3 (-35.1,16.6)	-9.2 (-33.2,14.9)

*Adjusted for age, sex, duration of follow-up, height (not for BMI), injury and tibial bone size
No interactions between sex and predictors on cartilage volume
BMI: body mass index
LBM: lean body mass



(a)



(b)

Figure 6.1. Association between body fat mass (a) and lean body mass (b) and tibial cartilage volume

6.3.3 Hormonal factors and tibial cartilage volume

SHBG measured in 78 female subjects 5 years prior was positively associated with total tibial cartilage volume in univariable and multivariable analysis (Table 6.3). Conversely, testosterone in females (n=78) showed a negative trend, but was not significantly, associated with tibial cartilage volume (Table 6.3). FAI was negatively associated with lateral tibial cartilage volume (β: -0.04 mm^3, 95% CI: -0.07, 0.00) and its association with total tibial cartilage volume was of borderline significance (p=0.053) (Table 6.3). All these association remained after further adjustment for fat mass.

Table 6.3. Association of sex hormones measured 5 years prior with total tibial cartilage volume

	Univariable	Multivariable*
Cartilage volume (mm^3)	β (95% CI)	β (95% CI)
Females		
SHBG#(nm/l)	**7.8 (2.3, 13.3)**	**0.67 (0.14, 1.20)**
Testosterone# (10fmol/l)	-17.5 (-49.5, 14.6)	-12.3 (-43.6, 19.0)
Free Androgen Index#	**-0.5 (-1.0, 0.0)**	-0.4 (-0.9, 0.1)

Measured only in females not taking oral contraceptives
*Adjusted for age, sex, BMI, duration of follow-up, injury and tibial bone size
No interactions between sex and predictors on cartilage volume
SHBG: sex hormone-binding globulin
Free androgen index was calculated as: testosterone/SHBG

6.3.4 Inflammatory factors and tibial cartilage volume

Fibrinogen measured 5 years prior was negatively associated with total tibial cartilage volume in univariable and multivariable analysis after adjustment for age, gender, BMI, duration, injury and tibial bone size (Table 6.4). CRP showed a negative trend associated with tibial cartilage volume, but this association did not reach the statistical significance in both univariable and multivariable analyses (Table 6.4). The significant association between fibrinogen and cartilage volume was only observed in males, but there were no interactions between sex and inflammatory markers on cartilage volume at p=0.1 level. The association of fibrinogen with cartilage volume became borderline significance (16% reduction in the effect size) after further adjustment for fat mass (p=0.064)

Table 6.4. Association of inflammatory markers measured 5 years prior with total tibial cartilage volume

	Univariable	Multivariable*
Cartilage volume (mm^3)	β (95% CI)	β (95% CI)
Total Sample		
CRP (mg/l)	-16.9 (-36.3,2.6)	-1.1 (-18.7,16.5)
Fibrinogen (g/l)	**-288.2 (-438.8, -137.6)**	**-146.4 (-276.4, -16.4)**
Females		
CRP (mg/l)	4.1 (-13.0, 21.2)	4.3 (-12.7, 21.3)
Fibrinogen (g/l)	-46.8 (-194.3, 100.8)	-69.3 (-227.3, 88.8)
Males		
CRP (mg/l)	-15.0 (-42.3, 12.2)	-18.7 (-64.7, 27.2)
Fibrinogen (g/l)	**-211.6 (-410.0, -13.2)**	**-219.7 (-432.9, -6.5)**

*Adjusted for age, sex, BMI, duration of follow-up, injury and tibial bone size
No interactions between sex and predictors on cartilage volume
CRP: c-reactive protein

6.3.5 Sex difference in tibial cartilage volume

As shown in Table 6.5, males had more tibial cartilage volume than females and sex explained 39% (R^2) of the variation in tibial cartilage volume in unadjusted analysis. In multivariable analysis after taking into account of factors including age, height, weight, duration of follow-up and bone size, males (4.11cm^3) had 13% more tibial cartilage volume than females (3.61cm^3). Sex was significantly associated with tibial cartilage volume (500 mm^3 difference) in multivariable analysis though the magnitude of coefficient reduced by 70%, and sex only explained 2.25% variation in tibial cartilage volume. The magnitude of sex difference in tibial cartilage volume decreased by 38% and became non-significant after further adjustment for LBM, and decreased by 20% but remained significant after further adjustment for total fat mass (Table 6.5). Both lean mass and fat mass explained almost all residual sex difference in tibial cartilage volume with sex explaining only 0.5% variation in tibial cartilage volume (Table 6.5). The magnitude of sex difference in tibial cartilage volume remained largely unchanged after adjustment for WHR or CRP (8% reduction), but decreased by 37% after adjustment for fibrinogen and the association became non-significant (Table 6.5).

Table 6.5. Sex difference in knee cartilage volume: mediating effects of body composition and inflammatory factors

Cartilage Volume (100mm^3)	β (95% CI)	R^2 or Partial R^2
Univariable	**12.48 (10.77, 14.20)**	38.6
Adjusted*	**3.68 (0.92, 6.44)**	2.25
Model 1	2.29 (-0.81, 5.38)	0.71
Model 2	**2.95 (0.16, 5.75)**	1.44
Model 3	-2.46 (-5.78, 1.31)	0.52
Model 4	**5.45 (2.14, 8.75)**	3.38
Model 5	**3.40 (0.53, 6.28)**	1.85
Model 6	2.33 (-0.52, 5.19)	0.90

*Adjusted for age, duration of follow-up, height, weight, injury and tibial bone size
Model 1: further adjustment for lean mass (instead of weight)
Model 2: further adjustment for fat mass (instead of weight)
Model 3: further adjustment for lean mass and fat mass (instead of weight)
Model 4: further adjustment for waist hip ratio (instead of weight)
Model 5: further adjustment for CRP
Model 6: further adjustment for fibrinogen

6.4 Discussion

To our knowledge, this is the first study to describe the factors associated with knee cartilage volume in young adults and to examine if these factors contribute to sex difference in tibial cartilage volume. We found that the lean mass was positively associated with tibial cartilage volume and explained 38% of the sex difference in tibial cartilage volume independent of other confounders. Conversely, fat mass, WHR and fibrinogen were negatively associated with tibial cartilage volume, and fat mass or fibrinogen explained significant part of sex difference in tibial cartilage volume. Furthermore, we explored the association of sex hormones with tibial cartilage volume in females and found that SHBG but not testosterone was positively associated with tibial cartilage volume.

In a young adult without OA, tibial cartilage volume is a measure of knee joint health. We have evidence to show that knee cartilage volume loss mainly starts (>2% per annum) at the age of 40 [183] and there was no significant difference in the loss of tibial cartilage volume between men and women before the age of 40 [206]. Subjects in our study were of mean age 36 and predominantly healthy and are expected to have a "peak" cartilage volume of their lifetime. Therefore, "peak" cartilage volume in younger adults, similar as peak bone mass that is a strong predictor of future risk of osteoporosis in older people [10], should be protective against the development of OA in later life. Above all, studies have found that increased knee cartilage volume is associated with reduced knee radiographic OA [7, 8], cartilage defects [8] and symptoms [9]. This suggests that understanding factors associated with "peak" knee cartilage volume in younger adults may be important in order to prevent the development of knee OA in later life.

Longitudinal studies have shown that BMI is associated with an increase in knee cartilage defects in healthy subjects [69]; however, the association of BMI with knee cartilage volume is controversial. Most of the studies failed to demonstrate an association of BMI with cartilage volume in osteoarthritic [70] or healthy knees [71, 72] although a few found that BMI was negatively associated with cartilage volume [62]. We reported that BMI was associated with knee cartilage loss only in people within the highest tertile of baseline knee cartilage volume [73]. These inconsistencies may be due to the inability of BMI to differentiate lean mass from fat mass. In this study we found that BMI at the time of MRI and 5 years prior was not associated with tibial cartilage volume; however, lean mass was positively and fat mass and WHR 5 years prior were negatively associated with tibial cartilage volume in these young adults. Similar results were observed longitudinally in healthy [207] and older adults [201] where lean mass was negatively and fat mass

positively associated with knee cartilage volume loss over time. Increased adipocytokines and other inflammatory markers produced as a result of the excess fat in the body might explain the negative association between fat mass and tibial cartilage volume. There is evidence to show that muscle mass [74] and muscle strength [75] are associated with more cartilage volume. Larger and stronger muscles might be protective for knee cartilage and helps to maintain the higher volume. The positive association between cartilage volume and skeletal muscle mass may in part reflect co-inheritance. However, this finding cannot completely be explained by body size, as we included body size in the model. Alternatively, this relationship may reflect common lifestyle or environmental factors such as physical activity, which similarly affect both cartilage and muscle [85]. It is also possible that muscle contributes to greater joint stability and even load distribution, which may in turn produce an optimal biomechanical environment that confers beneficial effects on cartilage [74].

The associations of sex hormones with knee OA are controversial. Cicuttini et al reported that the serum free testosterone level was positively associated with tibial cartilage volume in a cross-sectional study in healthy men;[62] however, serum free testosterone was associated with an increased rate of cartilage loss in the follow-up study [63]. We found that in females who were not taking oral contraceptives, testosterone measured 5 years prior was negatively but non-significantly associated with tibial cartilage volume. FAI, the active form of testosterone, was significantly and negatively associated with lateral tibial cartilage volume, suggesting a potentially detrimental effect of testosterone on knee cartilage volume in young women. Our results are in line with the longitudinal analysis of the previous study and may reflect the temporal relationship. The mechanism underlying the negative association between testosterone and tibial cartilage volume is unclear and needs further investigation.

A higher level of SHBG in women may reflect a higher estrogen levels or a lower androgen excess, glucocorticoid excess, or insulin resistance [208]. SHBG is more stable compared to sex hormones and there is an emerging recognition that low SHBG levels represent an independent biomarker of proinflammatory states associated with insulin resistance, and SHBG measurements may be a sensitive indicator of low-grade inflammation associated with the metabolic syndrome in obese individuals [209]. It is possible that lower levels of SHBG leads to a decreased cartilage volume through low-grade inflammation. We did not have estrogen measured in this sample, but found that SHBG was positively associated with tibial cartilage volume. A similar study reported a positive association between SHBG and patella bone volume, although they found no

associations with the tibial and patellar cartilage volume [64]. A potential physiological explanation of our findings is that SHBG is behaving as a surrogate marker for global estrogen exposure [64] and estrogen may be positively associated with the knee cartilage volume. Animal studies showed a protective effect of estrogen on cartilage and this effect was mostly seen in females [205]. This is supported by a clinical study, which reported that post-menopausal women using long-term estrogen replacement therapy had more knee cartilage than controls [210].

Inflammatory markers such as high-sensitivity CRP is independently and negatively associated with tibial cartilage volume in healthy women at mid-life suggesting that subclinical inflammation may predispose to knee tibial cartilage loss [202]. We have reported that inflammatory cytokines including IL-6 and TNF-alpha are associated with the cartilage loss;[203, 211] however, the association of fibrinogen with cartilage volume is unexplored. Fibrinogen, apart from its clotting function, functions as a messenger molecule that coordinates and regulates the inflammatory response; it is an acute phase protein and can be used as an inflammatory marker [212]. Fibrinogen was reduced after the weight loss in patients with knee OA although it didn't correlate with the symptom reduction. [213] Similarly, fibrinogen levels were reduced after supplementation of calcium fructoborate in knee OA patients [214]. We found that fibrinogen measured 5 years prior was negatively associated with tibial cartilage volume, but there was no significant association between CRP and tibial cartilage volume in younger adults, suggesting that specific types of inflammation are associated with reduced "peak" knee cartilage volume in young adults. Both males and females did not differ in terms of the direction of association of fibrinogen with tibial cartilage volume (no significant interaction between sex and fibrinogen on tibial cartilage volume). However, the association was only significant in males, and this may be due to slightly higher sample size and variability in cartilage volume in males. The association of fibrinogen and cartilage volume became borderline significance after including fat mass in the model indicating that this association is in part mediated by fat mass. Higher plasma levels of fibrinogen can induce a higher inflammation in synovial fluid and can promote cartilage degradation either directly or indirectly through their induction of proteolytic enzymes, amplifying a vicious cycle of innate immune activation.

It is well established that there is a sex difference in knee cartilage volume, but the factors that contribute to this difference are not clear. We found that in these younger adults, sex explained almost 39% of the variation in tibial cartilage volume. After adjustment for the covariates including body and bone size, sex only explained about 2.2% of the variation in tibial cartilage volume though this was still significant. In contrast, lean

mass and fat mass together explained almost all of the sex difference in tibial cartilage volume. Lean mass is the most important feature of body composition mediating this sex difference in cartilage volume (38% reduction in cartilage volume after adjustment for LBM). CRP had a small effect on the coefficient (8% reduction) of sex difference in cartilage volume; however, fibrinogen reduced the effect size of the sex difference by 37% and sex only explained 0.9% of the variation in tibial cartilage volume after adjustment for fibrinogen. Our study is the first to report that body composition and fibrinogen contribute to sex difference in knee cartilage volume.

A strength of our study was the use of a large representative sample with MRI to measure the tibial cartilage volume within a cohort study. This study had several potential limitations. First, the response rate was moderate with 43% of the participants having an MRI scan. Reassuringly, there were no significant differences in age, sex, BMI, and knee injury between those with and without MRI scans, or between subjects included in this study and the remainder of the original cohort, which suggests there was not major selection bias introduced. Second, fat mass and lean mass were calculated from skin-folds and may not be as accurate as dual-energy X-ray absorptiometry (DXA) measurement. However, the two correlate highly. Third, we measured sex hormones only in women who were not taking oral contraceptives and did not measure sex hormones in males and therefore were unable to examine if these sex hormones explained sex difference in tibial cartilage volume. Last, we did not have MRI scans at the time of obesity or blood measures 5 year prior; however, we are unlikely to see significant cartilage loss over time in this young population as knee cartilage volume starts to lose at the age of 40 [183].

In conclusion, factors including body composition, sex hormones and fibrinogen correlate with knee cartilage volume in young adult life suggesting potential for intervention. In addition, the sex difference in knee cartilage volume is contributed largely by variations in body composition and/or fibrinogen.

Chapter 7 - Association of physical activity and physical performance with tibial cartilage volume and bone area in young adults

7.1 Introduction

Knee osteoarthritis (OA) is characterised by the whole structural abnormalities including cartilage loss and subchondral bone changes along with clinical symptoms. Magnetic resonance imaging (MRI) has revolutionised the research in knee OA because of the clear visualisation of whole knee joint structures and the ability to manipulate the MR images to generate accurate data such as knee cartilage volume and tibial bone area [6]. Tibial cartilage volume measures the quantity of tibial cartilage covering the surface of tibial bone and tibial bone area is the cross-sectional surface area of the tibial plateau. Knee cartilage volume is lower in females and medial tibiofemoral compartment than in males [215] and lateral tibiofemoral compartment [216], respectively, which may explain why knee OA is more common in females and medial tibiofemoral compartment. Therefore, a low knee cartilage volume can be a risk factor for knee OA [62]. Similarly, bone size in a normal young adult is the result of genetics and adaptations to physical stimuli during skeletal growth [22], and increased tibial plateau in younger adults may be protective by reducing stresses in the joints [20], though tibial bone area has been associated with osteoarthritic changes in middle-aged and older adults [17].

Physical activity has been recommended to patients with knee and hip OA for improving their symptoms [143]. It is still controversial if physical activity has effects on joint structures. We reported that physical activity was beneficially associated with cartilage volume in healthy children [149, 151]. A systematic review has shown that various physical activity may have different effect on different knee structures [86], and there has been limited evidence that there is a positive relationship between physical activity and knee cartilage volume [86]. Most of the studies included in this review were done in older adults and only few have done in younger adults. It is possible that effects of physical activity on knee cartilage volume in younger adults are different from those in older adults. Physical activity increases the cortical bone size of tibia [217], which may influence the tibial surface area due to the adaptive mechanisms during growth.

While physical activity reflects a behavior, physical fitness is operationalised as several measurable health-related phenotypes including mainly cardiorespiratory fitness and muscle performance. There is a moderate correlation between physical activity and physical fitness measures and physical fitness is a better predictor of cardiovascular risk than physical activity [179]. Some studies focusing on physical performance measures (PPMs) such as muscle strength and physical fitness have shown a protective effect on knee structures [75, 91, 92]. It is unknown if physical activity and physical performance

have different effects on knee structures, particularly in young adults. Therefore, the aims of this study were to describe the associations of physical activity and physical performance measured 5 years prior with tibial cartilage volume and bone area in young adults.

7.2 Materials and Methods

7.2.1 Study participants

The Childhood Determinants of Adult Health (CDAH) Knee Cartilage study was conducted during 2008-2010. It was a follow-up study on a sub-sample (n=330, mean age 35, range 31-42) of participants in the CDAH study (n=2410, mean age 31). CDAH study was conducted during the period of 2004 to 2006 and included Australia-wide subjects aged between 26 to 31 years. Detailed descriptions of each of these studies are given in section 3.5. The CDAH participants were requested to have an MRI scan as part of the CDAH Knee Cartilage study. Two MRI scans were not readable and were excluded for the current analysis.

7.2.2 Anthropometric measurements

Weight was measured to the nearest 0.1 kg in CDAH study as well as in CDAH Knee Cartilage study with shoes, socks, and bulky clothing removed. Height was measured to the nearest 0.1 cm (with shoes and socks removed) using a stadiometer. Body mass index (BMI) was calculated as kilograms of weight per square metre of height.

7.2.3 Physical activity measurements:

Participants completed the long version of the International Physical Activity Questionnaire (IPAQ-L) in the CDAH study. Physical activity for leisure, transport, work and domestic purposes in the past week were assessed. Various domains of physical activities were considered including leisure and work related physical activity. Physical activities were calculated to represent min per week of vigorous physical activity, moderate physical activity, walking and total physical activity. Regular participation is a key concept included in current public health guidelines for physical activity [166]. Therefore, both the total volume and the number of day/sessions are included in the IPAQ analysis algorithms. Three levels of physical activity ‘low’, ‘moderate’, and ‘high’ are

developed from the IPAQ-L according to the guidelines (https://sites.google.com/site/theipaq/scoring-protocol; Guidelines for Data Processing and Analysis of the International Physical Activity Questionnaire). The IPAQ demonstrates very good levels of repeatability and fair to moderate validity when compared to accelerometer data [165].

7.2.4 Physical performance measures

PPMs including long jump, leg muscle strength and physical work capacity at 170 heart beats (PWC_{170}) were assessed in CDAH study.

Leg muscle strength was measured to the nearest 1.0 kg using dynamometers (TTM Muscle Meter, Tokyo, Japan). The dynamometer was adjusted to fit the size of each subject. Subjects were asked to stand on leg dynamometer with a straight back, leaning on the wall and holding the bar hand with an overhand grip. Knees were flexed to 115° at which the bar was attached to the dynamometer by a chain. Subjects kept a firm grip on the bar and pulled upward using only their legs, and keeping the back and neck straight, as far as possible. Subjects were instructed in each technique before testing and verbal encouragement was given until a maximal contraction was achieved. Two trials were recorded, with the mean score taken as the criterion value for leg strength. This test examines isometric strength of the whole legs, but predominantly of the quadriceps and hip extensors [162, 164].

Standing long jump was measured by asking subject to stand on the gym mat with toes behind the line and with feet slightly apart. A two-feet takeoff and landing was used, with the subject swinging arms and bending knees to provide the drive for the jump. The landing point at the closest part of the heel to the starting line was marked and the distance to the starting line was measured.

Cardiorespiratory fitness was estimated based on physical work capacity at a heart rate of 170 beats/min, which was assessed using a Monark bicycle ergometer [160]. Subjects were asked to cycle at a constant 60 rpm for 3 min each at three successively increasing but submaximal workloads. Heart rate was recorded at 1-min intervals at each workload using an electronic heart rate monitor. PWC_{170} was assessed by linear regression with extrapolation of the line of best fit to a heart rate of 170 beats/min. Repeatability was not assessed in our subjects but has previously been reported as an intraclass correlation coefficient (ICC) of 0.92 [161]. 261 subjects completed VO_{2max} test and the correlation between PWC_{170} and VO_{2max} was found to be 0.83 [162].

7.2.5 Tibial cartilage volume

Tibial cartilage volume was determined by means of 3D image processing on an independent work station using the software program OsiriX (Geneva, Switzerland). Individual plates of tibial cartilage volume (medial and lateral) were isolated by manually drawing disarticulation contours around the cartilage boundaries on a section-by-section basis. These data were then re-sampled by means of bilinear and cubic interpolation (area of 312 × 312 μm^2 and thickness of 1.5 mm, continuous sections) for the final 3D rendering. The coefficients of variation (CVs) for cartilage volume measures were 2.1-2.6% [7]. Total tibial cartilage volume was calculated as the sum of medial and lateral tibial cartilage volume.

7.2.6 Tibial bone area

The bone area of medial and lateral tibial plateau was measured manually on the three reformatted T1-weighted MR images closest to tibial cartilage in the axial plane as describe in the previous studies [7, 69, 173]. The CVs for these measures were 2.2-2.6% [7]. Total tibial bone area was calculated as the sum of medial and lateral area.

7.2.7 Statistical analyses

Mean and standard deviation or the percentages of the subjects were used for calculating the characteristics of the participants. Linear regression analyses were employed to examine the associations of physical activity and PPMs with adult tibial cartilage volume/bone area. Age at CDAH study, gender, duration of follow-up to CDAH Knee Cartilage study, BMI at CDAH Knee Cartilage study, knee injury and/or tibial bone area were examined as potential confounders. Interactions between sex and predictor variables were examined in the regressions of tibial cartilage volume/bone area. All statistical analyses were performed on SPSS 19 for Mac (SPSS Inc., Chicago, USA).

7.3 Results

7.3.1 Characteristics of the participants

Information on the demographic and study factors of the 328 study participants is presented in Table 7.1. Males had higher tibial cartilage volume, tibial bone area and PPMs such as PWC_{170}, leg muscle strength and long jump. Total physical activity, proportion of

IPAQ high activity category, knee injury and vigorous physical activity were also significantly greater in males than females. There were no significant differences in terms of age and BMI. There were no significant interactions between sex and predictor variables on tibial cartilage volume (data not shown) so we combined males and females for all analyses.

Table 7.1. Characteristics of the participants based on gender

	Female n=155	Male n=173	P-value
Age (yr)	35.3 (2.7)	35.4 (2.6)	0.275
Tibial cartilage volume (cm^3)	3.2 (0.7)	4.5 (0.9)	<0.001
Duration of follow-up (yr)	4.5 (1.2)	4.6 (1.2)	0.049
BMI (kg/m^2)	25.0 (4.6)	26.4 (3.7)	0.052
Tibial bone size (cm^2)	27.9 (2.4)	35.7 (3.3)	<0.001
Knee injury (%)	11.3 (n=17)	22.9 (n=40)	0.005
5 years prior			
IPAQ category (high%)	34.5 (n=53)	49.0 (n=85)	0.008
Total PA (min/week)	639.1 (403.1)	760.6 (566.3)	0.032
Vigorous PA (min/week)	95.6 (169.1)	195.5 (240.8)	<0.001
Moderate PA (min/week)	302.1 (275.6)	275.5 (241.7)	0.376
Walking (min/week)	239.2 (215.2)	289.6 (299.6)	0.098
PWC_{170} (per W)	138.5 (30.1)	202.6 (46.5)	<0.001
Leg muscle strength (per kg)	92.5 (26.6)	165.0 (34.3)	<0.001
Long jump (per cm)	141.9 (25.9)	190.7 (24.0)	<0.001

BMI: body mass index
TCV: tibial cartilage volume
PA: physical activity
IPAQ: international physical activity questionnaire
PWC_{170}: physical work capacity at 170 beats per minute
Two-tailed t tests used for differences between means; $\chi 2$ test used for proportions (percentages). Significant differences are shown in bold.
Mean (SD) except for percentages.

7.3.2 Physical activity and tibial cartilage volume and bone area

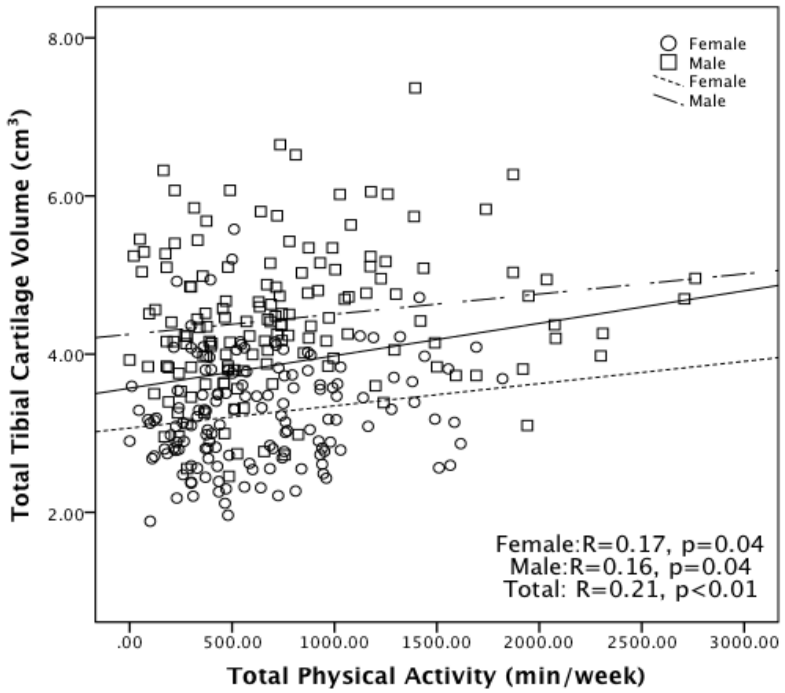
Total physical activity measured using the IPAQ-L 5 years prior was positively associated with tibial cartilage volume in univariable (Figure 7.1a) and multivariable analysis (Table 7.2). Vigorous physical activity, walking and the IPAQ category (low, moderate, high) were positively associated with tibial cartilage volume after adjustment for age, sex, BMI, injury, and duration of follow-up, and remained significant after further adjustment for tibial bone area (Table 7.2). The association between moderate physical activity and tibial cartilage volume became significant after further adjustment for tibial bone area (Table 7.2). Work related physical activity measured 5 years prior was also beneficially associated with tibial cartilage volume (β: 0.41 mm^3, 95% CI: 0.16, 0.66). These associations remained largely unchanged after further adjustment for the fitness measure PWC_{170}.

Total physical activity assessed 5 years prior was not significantly associated with tibial bone area (Figure 7.1b). None of these physical activity measures were significantly associated with tibial bone area in multivariable analysis after adjustment for age, sex, BMI, injury, and duration of follow-up (data not shown).

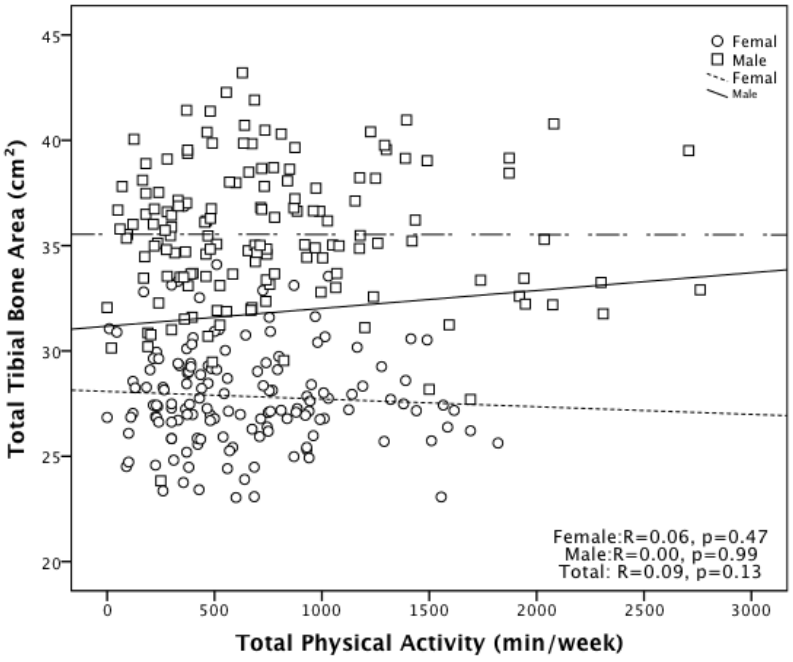
Table 7.2. Association between physical activities measured 5 years prior and total tibial cartilage volume

Cartilage Volume (mm^3)	Univariable β (95% CI)	Multivariable* β (95% CI)	Multivariable** β (95% CI)
IPAQ Category (high vs moderate and low)	**276.90 (103.42,450.39)**	**195.83 (56.27,335.38)**	**182.94 (51.84,314.04)**
Total PA (min/week)	**0.41 (0.17,0.63)**	**0.28(0.10,0.46)**	**0.30(0.13,0.47)**
Vigorous PA (min/week)	**1.18 (0.67,1.69)**	**0.55(0.12,0.98)**	**0.54(0.13,0.94)**
Moderate PA (min/week)	0.18 (-0.25,0.62)	0.30(-0.05,0.65)	**0.34(0.01,0.67)**
Walking PA (min/week)	**0.50 (0.07,0.93)**	**0.35(0.01,0.069)**	**0.40(0.07,0.72)**

*Adjusted for age, sex, duration of follow-up, BMI, knee injury.
** Further adjusted for tibial bone area
IPAQ: International Physical Activity Questionnaire
PA: Physical Activity
Bold denotes statistical significance at $p<0.05$



(a)



(b)

Figure 7.1. Association of total physical activity with total tibial cartilage volume (a) and tibial bone area (b)

7.3.3 Physical performance measures and tibial cartilage volume and bone area

All PPMs were positively associated with tibial bone area in univariable analysis (Figure 7.2a, Table 7.3). These associations remained significant after adjustment for age, sex, BMI, injury and duration of follow-up (Table 7.3). Adjusting for physical activity levels did not make any difference to these associations (data not shown).

All PPMs including PWC_{170} (Figure 7.2b) were positively associated with tibial cartilage volume in univariable analysis (Table 7.4). These associations remained significant after adjustment for age, sex, BMI, knee injury and duration of follow-up. The magnitudes of these associations decreased by 30-47%, and the association of leg muscle strength became of borderline significance after further adjustment for tibial bone area (Table 7.4). These associations remained unchanged after further adjustment for total physical activity (data not shown).

Table 7.3. Association between physical performances measured 5 years prior and total tibial bone area

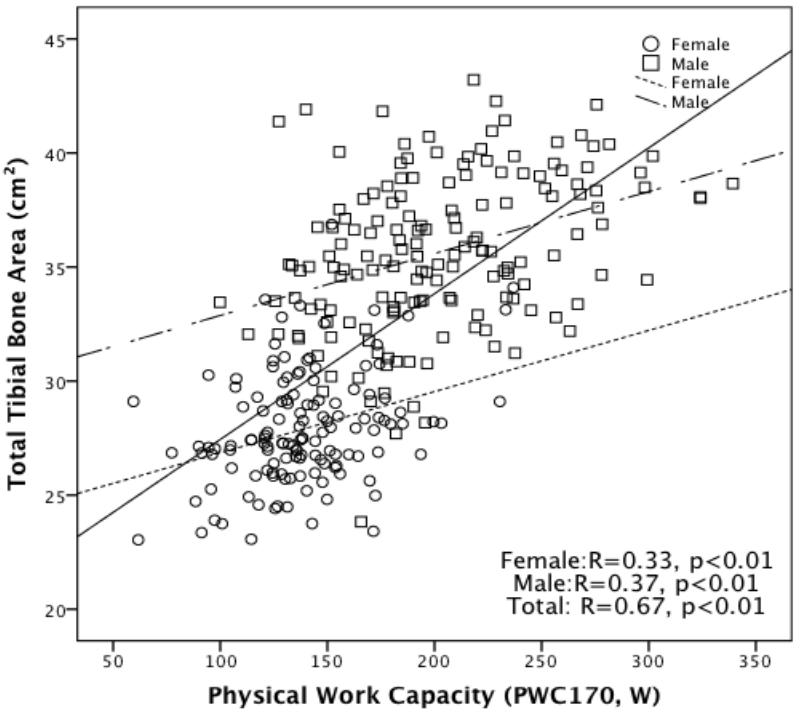
	Univariable	Multivariable*
Bone Area (mm^2)	β (95% CI)	β (95% CI)
Long Jump (per cm)	**8.51(7.20,9.83)**	**1.94(0.44,3.45)**
PWC_{170} (per W)	**6.39(5.57,7.21)**	**2.83(2.00,3.66)**
Leg muscle strength (per kg)	**7.18(6.35,8.01)**	**2.17(1.07,3.26)**

*Adjusted for age, sex, duration of follow-up, BMI, knee injury
Bold denotes statistical significance at $p<0.05$

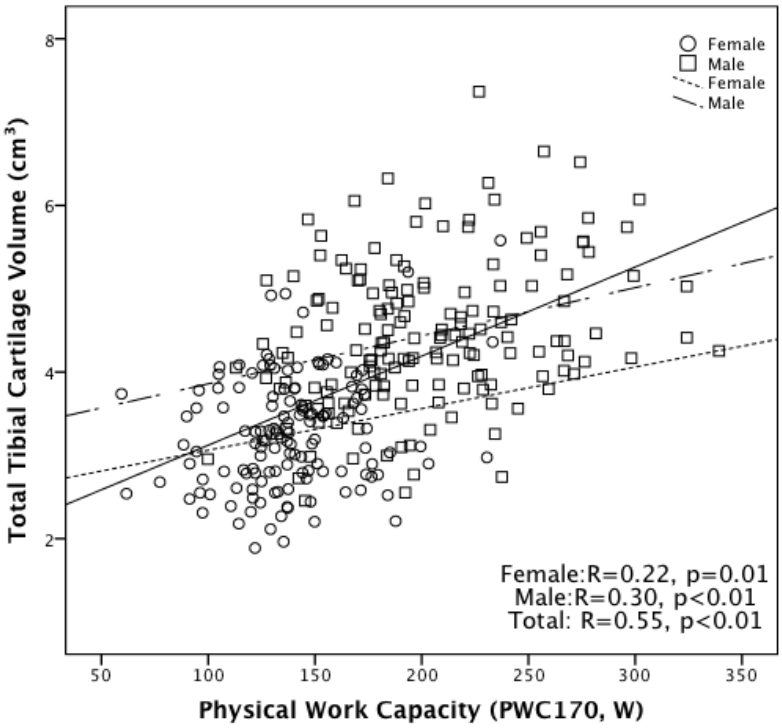
Table 7.4. Association between physical performances measured 5 years prior and total tibial cartilage volume

	Univariable	Multivariable*	Multivariable**
Cartilage Volume (mm^3)	β (95% CI)	β (95% CI)	β (95% CI)
Long Jump (per cm)	**14.85 (11.99,17.70)**	**6.06(2.14,9.98)**	**4.26 (0.54,7.97)**
PWC_{170} (per W)	**10.69 (8.83,12.56)**	**5.71(3.46,7.96)**	**3.45 (1.13,5.77)**
Leg muscle strength (per kg)	**11.53 (9.55,13.51)**	**4.16(1.24,7.09)**	2.20 (-0.63,5.03)

*Adjusted for age, sex, duration of follow-up, BMI, knee injury
** Further adjusted for bone area
Bold denotes statistical significance at $p<0.05$



(a)



(b)

Figure 7.2. Association of PWC_{170} with total tibial bone area (a) and total tibial cartilage volume (b)

7.4 Discussion

To our knowledge, this is the first study that explored the association of physical activity and physical performance with knee cartilage volume and bone area in young adults aged 31-41 years. We found that the physical activity and PPMs were positively associated with tibial cartilage volume and the association of PPMs with tibial cartilage volume was partly mediated by bone area. While PPMs were significantly associated with tibial bone area, there was no significant association between physical activity and tibial bone area.

Tibial cartilage volume is measured using MRI technique and it is associated with structural and functional characteristics of knee OA. Participants in our study were of mean age 36 and cartilage volume loss usually starts around the age of 40 [183]. Therefore, these predominantly healthy subjects are expected to have "peak" cartilage volume of their lifetime. "Peak" cartilage volume in the absence of other knee structural abnormalities predominantly reflects a healthier cartilage. Similar to the peak bone mass that is a strong predictor of future risk of osteoporosis in older people [10], "peak" cartilage volume in a younger age group should be protective against the development of OA in later life. Further more, studies suggest that increased cartilage volume is associated with reduced radiographic OA [7, 8], cartilage defects [8] and knee symptoms [9].

Tibial bone area or the cross-sectional tibial plateau surface areas is a dynamic structure that may respond to physical stimuli [181]. Tibial bone area is positively associated with cartilage defects and cartilage volume loss in older adults [17] and has been shown to be an independent predictor of knee replacement over 4 years [19]. Tibial bone area increase in older populations appears maladaptive and is possibly due to disproportionate load transmission on the bone [20]. In contrast, exercise during childhood and adolescence is associated with increased cortical bone size which persists over years leading to increased bone mass [21, 22]; therefore, increased bone area during growth may reflect adaptive change. Hypothetically, a greater tibial plateau will limit stresses in the joints because higher loads are distributed over a greater surface [23]. This is consistent with the findings from a cross-sectional study in which tibial bone area was larger in athletes than in controls [20].

We have previously reported that physical activity in children with no injury was associated with increased cartilage volume cross-sectionally and longitudinally [149, 150]. The most striking association was with vigorous activity in the last two weeks: children with any vigorous activity had 22–25% more cartilage volumes compared with those

without vigorous activity. Racunica et al reported that tibial cartilage volume increased with frequency and duration of vigorous activity reported 10 years previously, as well as recent vigorous activity in the 7 days prior to MRI in healthy, community-based adults with no history of knee injury or disease [85]. Another study found that in the subgroup with no significant patella cartilage defects at baseline, participation in vigorous physical activity was associated with a reduced annual loss of patellar cartilage volume and a trend for fewer new patella cartilage defects [145]. Participation in exercise that causes tachypnea and an increased pulse rate for at least 20 minutes was associated with greater medial tibial cartilage volume in non-healthcare-seeking women at midlife [218]. In contrast, a cross-sectional study of young adult triathletes reported no difference in knee cartilage volume between triathletes and inactive volunteers, although knee bone area was larger in triathletes [20]. Another cross-sectional study in healthy older men found that physical activity was negatively associated with medial tibial cartilage volume [62]. Both these studies were small in sample size; however, in older subjects with abnormalities in knee joint such as bone marrow lesions or lower cartilage volume, persistent participation in vigorous physical activity was associated with adverse effects on cartilage in the medial compartment [145, 219, 220]. All these suggest that the effects of vigorous physical activity on knee structures may depend on age or the preexisting health of the joint.

The prevalence of structural abnormalities in this healthy young adult sample was very low (<15%) and we found that all the physical activity measures including vigorous, moderate and total physical activity measured 5 years prior were positively associated with tibial cartilage volume. This was largely independent of tibial bone area and fitness levels suggesting that physical activity is beneficial to maintain healthy knee cartilage in younger adults. This can be explained in line with the studies that reported a positive effect of moderate exercise on glycosaminoglycan content in knee cartilage using delayed Gadolinium Enhanced MRI of Cartilage (dGEMRIC) assessment [152, 153]. It was also noted that there was no significant association between any of the physical activity measures and tibial bone area suggesting that tibial bone area may not be as responsive as tibial cartilage volume to physical stimuli in younger adults.

Muscle-strengthening intervention is now a major component of the usual treatment programme for patients with knee OA, despite the little information regarding their effects on disease progression. Studies reported that increased muscle mass was associated with medial tibial cartilage volume and a reduction in the loss of tibial cartilage [97] and physical fitness especially PWC_{170} was associated with a reduced cartilage volume loss over time and was positively associated with change in tibial bone area at lateral and total

sites [75]. In the current study, we found that PWC_{170}, leg muscle strength and long jump were positively associated with tibial cartilage volume. Performance measures, especially PWC_{170} were strongly correlated (r=0.55) with cartilage volume than physical activity (r=0.21). This could be partly due to the objective measurement of PWC_{170} measure compared to subjective measurement of physical activity by questionnaire. Additionally, physical performance measures and cartilage volume may share genetic components while physical activity does not. This is evident from the finding that PWC_{170} was more strongly correlated with tibial bone area (r=0.67) and physical activity was not (r=0.09). We also found that tibial bone area partly mediated the association between PPMs and cartilage volume. These results were consistent with our previous findings in children where leg muscle strength was positively associated with cartilage volume [151] and tibial bone area (unpublished data). We also found that childhood physical performance measures, especially PWC_{170}, was positively associated with adulthood tibial bone area and cartilage volume independent of the adulthood fitness levels [221]. These associations of childhood PPMs with adult knee cartilage volume were also partially mediated by tibial bone area [221], suggesting that the higher PPMs lead to higher knee cartilage volume in part through the development of knee bone area.

A strength of our study was the use of 5-year prospective data with physical activity and PPMs at baseline and tibial cartilage volume/bone area after 5 years. This study has several potential limitations. The response rate was low with only 43% of the persons invited to participate having MRI performed. Reassuringly, there were no significant differences in age, sex, BMI, and knee injury between those with and without MRI scans, or between subjects included in this study and the remainder of the original cohort, which suggests there was not major selection bias introduced. We did not measure knee malalignment in this study and could not account for the influence of alignment on cartilage volume. Physical activity and performance measures were assessed 5 years prior and we did not have MRI measurements at that time. We have previously reported the loss of cartilage volume starts at the age of 40. Therefore, it is expected that our subjects may have a similar cartilage volume 5 years prior.

In conclusion, while tibial bone area is affected only by physical performance, tibial cartilage volume can be influenced by both physical activity and physical performance in younger adults. The clinical significance suggests a beneficial effect of activity for cartilage but the bone area association was restricted to performance suggesting other factors rather than physical activity may be important.

Chapter 8 - Correlates of knee bone marrow lesions in younger adults

8.1 Introduction

Osteoarthritis (OA) affects whole joint structure including subchondral bone that results in joint pain and dysfunction. Subchondral bone is rich in blood and nerve supply and there is emerging evidence to suggest that changes in bone precede cartilage damage, so that bone rather than cartilage may be the site initiating the significant pathophysiological events in OA [222]. Subchondral bone marrow lesions (BMLs) play a key role in the pathogenesis of OA [223] and are associated with pain [29]. BMLs can regress and progress over time [42] and BML regression is associated with a decrease in knee pain [35, 36]. This makes BML an attractive target for treatment of OA [37] and there have been recent clinical trials [26]. BMLs in the tibiofemoral joint can predict both structural and clinical changes in knee OA [24, 224] and the natural history of BMLs has been explored in older adults [225]. Little is known about their determinants and clinical significance in young populations.

The prevalence of vascular disease is high among people with OA [226]. These diseases may share some common risk factors such as obesity, high low-density lipoprotein (LDL) levels and elevated total cholesterol [227]. Subchondral bone ischaemia may be one of the mechanisms by which vascular pathology contributes to the development of BMLs [228]. Total cholesterol and triglycerides were associated with BMLs in women [229] and high-density lipoprotein (HDL) cholesterol seems to have a protective effect on the incidence of BMLs in older adults [228]. Again, these studies are mostly performed in older adults and there are no studies in young adults.

The association of BMLs with physical activity is controversial. The current evidence, mostly in older adults, is mixed with some showing beneficial [85] and some detrimental effects [220, 230]; a recent systematic review found limited evidence overall for an association between physical activity and BMLs [231]. The associations between physical activity and BMLs in young adults have not been explored.

The aims of this study were, therefore, to examine the environmental, structural and clinical correlates of BMLs in younger adults and to determine whether lipid levels measured 5 years prior were associated with current BMLs in young adults.

8.2 Materials and Methods

8.2.1 Study population

Participants (n=330, aged 31-41 years, female 48.7%) were broadly representative of the Australian population because they were originally part of the Australian Schools Health and Fitness Survey (ASHFS) of 1985. The Childhood Determinants of Adult Health (CDAH) Knee Cartilage study was conducted during 2008-2010. It was a follow-up study on a sub-sample (n=330, mean age 35, range 31-42) of participants in the CDAH study (n=2410, mean age 31). CDAH study was conducted during the period of 2004 to 2006 and included Australia-wide subjects aged between 26 to 31 years. Detailed descriptions of each of these studies are given in section 3.5. The CDAH participants were requested to have an MRI scan as part of the CDAH Knee Cartilage study. Two MRI scans were not readable and were excluded for the current analysis.

8.2.2 Anthropometric measurements

Weight was measured to the nearest 0.1 kg in CDAH study as well as in CDAH Knee Cartilage study with shoes, socks, and bulky clothing removed. Height was measured to the nearest 0.1 cm (with shoes and socks removed) using a stadiometer. Body mass index (BMI) was calculated as kilograms of weight per square metre of height.

8.2.3 Physical activity measurements

Subjects in the CDAH Knee Cartilage study completed a short version of the International Physical Activity Questionnaire (IPAQ). Physical activities were calculated to represent minutes per week of vigorous physical activity, moderate physical activity, walking and total physical activity. Regular participation is a key concept included in current public health guidelines for physical activity [166]. Therefore, both the total volume and the number of day/sessions are included in the IPAQ analysis algorithms. The IPAQ demonstrates very good levels of repeatability and fair to moderate validity when compared to accelerometer data [165].

8.2.4 Knee symptom measures

Knee symptoms during the past 30 days were assessed using the Western Ontario and McMaster University osteoarthritis index (WOMAC) scale in CDAH Knee Cartilage

study. Subjects were asked about the knee pain, stiffness and physical dysfunction status during computer assisted telephone interview. Each question was graded on a scale of 0-9, where 0 indicated no symptoms and 9 indicated the maximum intensity of the symptoms. The WOMAC is an established scale for OA research and is also validated for responsiveness of knee complaints in young population without OA [169]. Total WOMAC scores were calculated by adding the scores of 5 subscales in WOMAC knee pain, 2 subscales in WOMAC stiffness and 17 subscales in WOMAC dysfunction. The presence of any pain, stiffness and dysfunction was defined as any score ≥1. Total WOMAC knee pain was also categorised into two groups based on total WOMAC score of >5 and ≤5.

8.2.5 Cholesterol measures

Venous blood samples were collected from the antecubital vein after a 12-hour fast in CDAH study approximately 5 years prior to CDAH Knee Cartilage study. Serum total cholesterol and HDL cholesterol concentrations were determined enzymatically (Olympus AU5400 automated analyzer, Olympus Optical, Tokyo, Japan) and the Lipid Research Clinic procedures were followed. LDL cholesterol concentration was calculated using the Friedewald formula [168].

8.2.6 BML measurement

BMLs were measured using the coronal proton density-weighted images and was marked as the increased signal intensity area in the subchondral bone adjacent to the osteochondral junction. BMLs were scored in medial and lateral compartment of tibia and femur using an ordinal scoring system which we used previously [24]. Subjects with no BMLs were scored as grade 0 and then the subjects with BMLs were graded according to the percentage of area of occupancy of BML in each compartment: grade 1: ≤25% of area; grade 2: >25%<50%; grade 3: >50%.

8.2.7 Cartilage defects

Cartilage defects were measured using both proton density coronal images and T1-weighted sagittal images. Cartilage defects were graded in an ordinal scale as we published before [8]. Grade 0 indicated a normal cartilage, grade 1 indicated focal blistering and low (T1-weighted) or high (proton density-weighted) signal intensity area with intact surface/bottom. Grade 2 indicated a loss of thickness of less than 50% on surface/bottom

of the cartilage. Grade 3 represented a deep ulceration with loss of thickness >50%, and grade 4 indicated a full-thickness chondral wear with exposure of subchondral bone. A prevalent cartilage defect was defined as a cartilage defect score of ≥2 at any site within that compartment.

8.2.8 Meniscal tear

Meniscal tear was graded in medial and lateral menisci separately based on a combined Whole-Organ Magnetic Resonance Imaging Score (WORMS) scoring system [174] from grade 0 to 2 using proton density-weighted coronal and T1- weighted sagittal images. Grade 0 was a fully normal intact meniscus. Grade 1 indicated a non-displaced tear (scored as grade 1 and 2 by WORMS). Grade 2 indicated a displaced tear or maceration (scored as grade 3 and 4 by WORMS).

8.2.9 Meniscal extrusion

Meniscal extrusion was recorded on the medial and lateral menisci and was graded from grade 0 to 2 based on the proton density-weighted coronal images as published before [175]. Grade 0 was an intact meniscus without any degree of extrusion. Grade 1 indicated a partially displaced meniscus with respect to tibia and Grade 2 represented a completely displaced meniscus. Meniscal lesions were defined as any meniscal tear or meniscal extrusion in the knee.

8.2.10 Statistical analyses

Mean and standard deviation or the percentages of the subjects were used for calculating the characteristics of the participants. T- test or chi- square test were used to compare the characteristics of the participants based on the presence or absence of any BML. Univariable and multivariable log binomial regression was used to estimate prevalence ratio (PR) of the associations between any BML and knee symptoms, structural pathologies, physical activity and cholesterol before and after adjustment for potential confounders. If the log binomial model failed to converge, PR was estimated using a Poisson distribution and robust standard errors. Age, gender, BMI, previous knee injury and/or duration of follow-up (for cholesterol analyses only) were examined as potential confounders based on the significant associations with BMLs or our previous literature

suggesting that these are important covariates. All statistical analyses were performed on SPSS 19 for Mac (SPSS Inc., Chicago, USA).

8.3 Results

8.3.1 Characteristics of the participants

The prevalence of any BML in the knee joint was 17%. The baseline characteristics of the participants based on the presence or absence of BMLs are presented in Table 8.1. Participants with any BML were similar in terms of gender distribution, BMI, total physical activity and total and LDL cholesterol when compared with participants without BMLs. However, participants with BMLs were older, had lower moderate physical activity, higher proportion with cartilage defects, meniscal lesions, and WOMAC pain, stiffness and dysfunction. HDL cholesterol was lower in participants with BMLs compared to those without BMLs.

Table 8.1. Baseline characteristics of the participants based on their bone marrow lesion status

	No BML (n=272)	**Any BML** (n=55)	**P-value**
Age (years)	35.2(2.7)	36.0(2.6)	0.053
Sex (male, %)	52 (n=141)	58 (n=32)	0.239
BMI (kg/m^2)	25.7(4.3)	25.6(4.0)	0.864
Knee Injury (%)	16 (n=41)	24 (n=13)	0.113
Total Physical Activity (min/week)	927.4(1308.9)	932.6(1354.1)	0.979
Total Vigorous PA (min/week)	205.2(446.6)	349.0(987.9)	0.094
Total Moderate PA (min/week)	**277.3(621.6)**	**122.7(189.2)**	**0.001**
Cartilage Defects (%)	**4.4 (n=12)**	**16.4 (n=9)**	**0.003**
Total Meniscal Extrusion (%)	**3.3 (n=9)**	**11.1 (n=6)**	**0.024**
Total Meniscal Tear (%)	12 (n=33)	22 (n=12)	0.051
Total Meniscal Lesions (%)	**13.6 (n=37)**	**27.3 (n=15)**	**0.013**
WOMAC pain (yes, %)	34 (n=90)	45 (n=25)	0.084
WOMAC stiffness (yes, %)	31 (n=80)	41 (n=23)	0.075
WOMAC dysfunction (yes, %)	**39 (n=101)**	**56 (n=30)**	**0.018**
WOMAC Pain (≥5 vs <5, %)	**11 (n=29)**	**22 (n=12)**	**0.032**
HDL cholesterol (fasting, mmol/L)	**1.45(0.34)**	**1.34(0.28)**	**0.019**
LDL cholesterol (fasting, mmol/L)	2.90(0.80)	2.87(0.69)	0.727
Total cholesterol (fasting, mmol/L)	4.86(0.93)	4.69(0.74)	0.145

BMI: body mass index
PA: physical activity
HDL: high-density lipoprotein
LDL: low-density lipoprotein
WOMAC: Western Ontario and McMaster Universities osteoarthritis index
Two-tailed t tests used for differences between means; $\chi 2$ test used for proportions (percentages).
Mean (SD) except for percentages.

8.3.2 Physical activity and BMLs

Cross-sectional associations between demographic factors, physical activity and BMLs are shown in Table 8.2. Age was associated with any BML in univariable and multivariable analysis. Gender, BMI, and previous knee injury were not significantly associated with any BML; however, previous knee injury was associated with medial tibiofemoral BMLs (PR: 2.20, 95% CI: 1.03, 4.71) in multivariable analysis. Total physical activity was not associated with any BML in the knee, but moderate physical activity was protectively associated with any BML before and after adjustment for age, gender, BMI and knee injury. Vigorous physical activity showed a weak deleterious association with medial tibiofemoral BMLs (PR: 1.02, 95% CI: 1.01, 1.03) and the association with any knee BMLs was of borderline significance.

Table 8.2. Associations of demographic factors and physical activity with bone marrow lesions in young adults

	Univariable PR(95% CI)	**Multivariable* PR(95% CI)**
Age (per year)	**1.09(1.00, 1.20)**	**1.10(1.00,1.20)**
Sex (females vs males)	1.24(0.76,2.02)	1.26(0.76,2.08)
BMI (per kg/m^2)	1.00(0.94,1.05)	0.99(0.93,1.05)
Knee Injury (yes vs no)	1.50(0.87,2.60)	1.49(0.86,2.58)
Total Physical Activity (per hour/week)	1.00(0.99,1.01)	1.00(0.99,1.01)
Total Vigorous PA (per hour/week)	**1.01(1.00,1.02)**	1.01(0.99,1.02)
Total Moderate PA (per hour/week)	**0.94(0.88,0.99)**	**0.93(0.87,0.99)**

*Adjusted for age, sex, BMI, and knee injury
PR: prevalence ratio
95% CI: 95% confidence interval
BMI: body mass index
PA: physical activity

8.3.3 Cholesterol measures and BMLs

HDL cholesterol measured approximately 5 years prior was negatively associated with any BML in univariable and multivariable analysis (Table 8.3). Figure 8.1 shows the prevalence of BMLs based on quartiles of HDL cholesterol. Total cholesterol or LDL cholesterol was not significantly associated with any BML of the knee after adjustment for age, gender, BMI, duration of follow-up and knee injury (Table 8.3).

Table 8.3. Association of cholesterol measured approximately 5 years prior with bone marrow lesion

	Univariable PR(95% CI)	Multivariable* PR(95% CI)
As continuous variables		
HDL cholesterol (per mmol/L)	**0.42(0.20,0.88)**	**0.36(0.15,0.87)**
LDL cholesterol (per mmol/L)	0.95(0.71,1.27)	0.94(0.69,1.27)
Total cholesterol (per mmol/L)	0.83(0.65,1.07)	0.84(0.66,1.06)

*Adjusted for age, sex, BMI, duration of follow-up and knee injury
PR: prevalence ratio
95% CI: 95% confidence interval
HDL: high-density lipoprotein
LDL: low-density lipoprotein

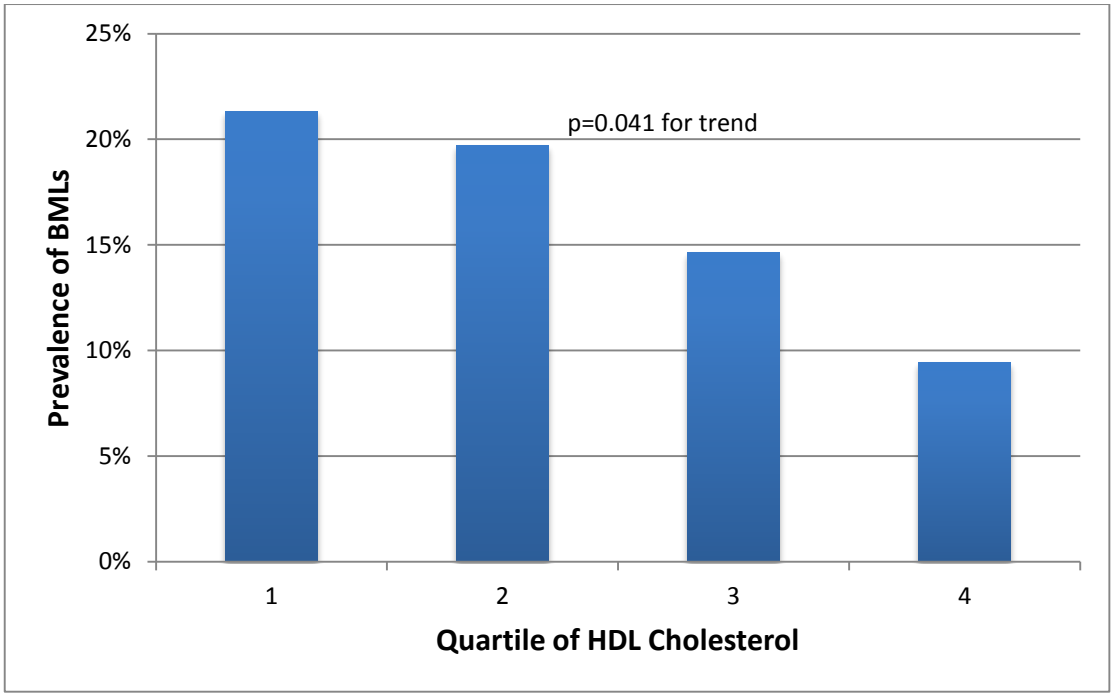


Figure 8.1. Associations between prevalence of any bone marrow lesion and quartile of high-density lipoprotein cholesterol measured approximately 5 years prior.

p-value from multivariable log binomial regression after adjusting for age, sex, body mass index, knee injury and duration of follow-up
BML: bone marrow lesion
HDL: high-density lipoprotein

8.3.4 Knee symptoms and structural lesions with BMLs

Figure 8.2 shows the prevalence of BMLs in each pain category based on the grouping of total WOMAC pain (0, ≥1 to 5, >5). Any BML in the knee was not significantly associated with any total WOMAC knee pain (≥1 vs =0); however, when we categorised total WOMAC knee pain as >5 vs ≤5 for a severity analysis, there was a significant association with BMLs before and after adjustment for covariates (Table 8.4). Any BML was also associated with any WOMAC dysfunction in univariable and multivariable analyses (Table 8.4).

As shown in Table 8.4, BMLs were associated with other knee structural abnormalities including total knee cartilage defects and total meniscal extrusion, as well as total meniscal lesions defined as any tear or extrusion of the menisci. BMLs were not significantly associated with cartilage volume or bone area in these young adults (data not shown).

Table 8.4. Association of bone marrow lesions with knee symptoms and structural abnormalities

	Univariable PR(95% CI)	**Multivariable* PR(95% CI)**
Symptoms		
WOMAC Pain (any vs no)	1.46(0.90,2.35)	1.41(0.86,2.33)
WOMAC Stiffness (any vs no)	1.49(0.92,2.40)	1.48(0.90,2.45)
WOMAC Dysfunction (any vs no)	**1.75(1.07,2.84)**	**1.75(1.07,2.89)**
WOMAC Pain (>5 vs ≤5)	**1.87(1.08,3.24)**	**1.80(1.03,3.14)**
Structural abnormalities		
Cartilage Defects (any vs no)	**2.85(1.63,4.50)**	**2.54(1.49,4.34)**
Meniscal Extrusion (any vs no)	**2.59(1.32,5.08)**	**2.50(1.29,4.82)**
Meniscal Tear (any vs no)	**1.75(1.00,3.05)**	1.63(0.93,2.86)
Meniscal Lesion (any vs no)	**1.98(1.19,3.32)**	**1.92(1.13,3.28)**

*Adjusted for age, sex, BMI, and knee injury
PR: prevalence ratio
95% CI: 95% confidence interval
WOMAC: Western Ontario and McMaster Universities osteoarthritis index

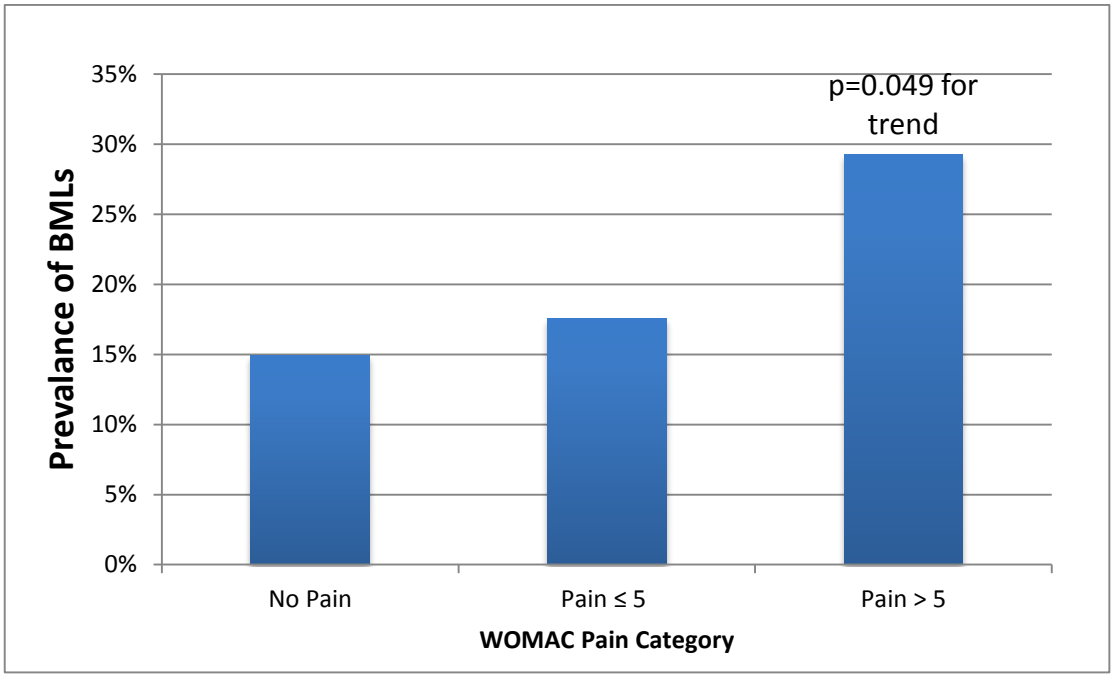


Figure 8.2. Association between prevalence of any bone marrow lesion and category of total knee pain (0-45).

p-value from multivariable log binomial regression after adjusting for age, sex, body mass index and knee injury
BML: bone marrow lesion
WOMAC: Western Ontario and McMaster Universities osteoarthritis index

8.4 Discussion

This is the first study exploring the correlates of knee BMLs in a population-based sample of young adults. Knee BMLs were surprisingly commonly being found in 17% of participants. They were associated with increasing age, previous knee injury, increased knee symptoms and structural abnormalities such as meniscal lesions and cartilage defects. Furthermore, moderate physical activity and higher HDL cholesterol were associated with decreased while vigorous activity was weakly related with increased BML, suggesting that BMLs in young adults are modifiable.

BMLs are commonly found in knee OA patients and are also present in non-OA populations. Prevalence of BMLs in this young population-based cohort was 17%, which is comparable with the prevalence reported previously in an older but non-symptomatic or non- OA population [39, 40, 232]. Pathology of BMLs are complex and BML zone mainly consisted of normal tissue (53% of the area was fatty marrow, 16% was intact trabeculae, and 2% was blood vessels) and a smaller proportion of several abnormalities (bone marrow necrosis {11% of area}, abnormal (necrotic or remodeled) trabeculae {8%}, bone marrow fibrosis {4%}, bone marrow oedema {4%}, and bone marrow bleeding {2%}) [233]. Subchondral bone is rich in blood supply and it is possible that the systemic and local factors can play a role in the BML pathology. We found that age was associated with increased risk of BMLs, which is consistent with a finding in a non-OA population cohort [38]. Injury was associated with medial tibiofemoral BMLs, which is in line with the finding observed in middle-aged and older adults [41]. However, BMI was not significantly associated with BMLs in this young cohort. Similar results were also observed in healthy middle-aged and older adults [38, 74]. However, a systematic review has suggested a moderate level of evidence for the association between obesity measures and BMLs [234].

The current evidence of the association of physical activity with BMLs are mostly in older adults and suggests beneficial [85], detrimental [220, 230, 235] or no effect [38, 146]. Racunica et al reported that non-vigorous activity (less vigorous activity and walking) was negatively associated with BMLs [85] in healthy older adults; in contrast, we reported that strenuous physical activity independently predicted an increase in BML size in middle-aged adults [42]. Similarly, we found that doing ≥10000 steps per day was associated with an increase in BML in older adults [220]. Asymptomatic middle-aged individuals from the “Osteoarthritis Initiative” incidence cohort had more knee BMLs in those who were more physically active, independent of confounders [230]. We found in

this young population that although total physical activity was not associated with BMLs, moderate physical activity was associated with reduced BMLs in the whole knee but vigorous physical activity was weakly associated with increased BMLs in medial tibiofemoral compartment. These findings suggest that different levels of physical activity may have different influences on subchondral bone health in young adults.

Vascular pathology is proposed in the pathophysiology of OA. Increased popliteal artery wall thickness was associated with reduced medial tibial cartilage volume, increased rate of cartilage volume loss and a trend for BML worsening over 2 years [236]. Total cholesterol and triglycerides were associated with increased BMLs in women [229]. In our study, we found that although total cholesterol and LDL measured 5 years prior were not associated with BMLs, HDL cholesterol measured 5 years prior was significantly associated with reduced BMLs. This is consistent with our previous longitudinal findings in older adults where HDL cholesterol was associated with BML resolution and had a protective effect on incidence of BMLs [228]. HDL cholesterol is considered to be protective against vascular pathology through cholesterol transport, anti-inflammatory, and antioxidant effects, and therefore, may help to reduce BML development and progression [237].

Subchondral bone is richly innervated with nociceptive pain fibers [28]. Thus, subchondral bone could be a source of knee pain. There is increasing evidence to suggest that BMLs are associated with knee pain [29-32] in older adults or knee OA patients, though some studies did not establish this association [33, 34]. A systematic review suggested that there was moderate evidence for the association between BMLs and knee pain [12]. We found that BMLs were associated with more severe knee pain when the total WOMAC pain was categorised at 5 in young adults, suggesting that BMLs in young adults would also be a source of knee pain.

Structurally, BMLs appear to be sclerotic compared with unaffected regions from the same individual based on the increased bone volume fraction and increased trabecular thickness [238, 239]. The mineral density in these lesions, however, is reduced and may render this area to be mechanically compromised, and thus susceptible to attrition [238]. Therefore, it is highly likely that BMLs can have a local mechanical effect on the knee joint. It has been reported that BMLs can predict site-specific cartilage pathology including cartilage defect progression and cartilage volume loss in older [24, 240] and middle-aged [232] adults. Similarly, BMLs are associated with meniscal pathology [241] and meniscal pathology increases the risk for the incidence and progression of BMLs in older adults [242]. This study is the first to report that in young adults, BMLs were associated with

meniscal lesions and cartilage defects. We did not find any significant association with knee cartilage volume or bone area, which concurs with the results from a study in healthy middle-aged women [39], indicating that BMLs may be associated with earlier structural changes such as cartilage defects rather than later structural changes such as reduced cartilage volume in young populations.

A strength of our study was the use of a population-based sample in young adults. This study has several potential limitations. The response rate was low with only 43% of the persons invited to participate having MRI performed. Reassuringly, there were no significant differences in age, sex, BMI, and knee injury between those with and without MRI scans, or between subjects included in this study and the remainder of the original cohort, which suggests there was no major selection bias introduced. Cholesterol measures were assessed 5 years prior and we did not have MRI measurements at that time, so cannot examine if cholesterol was associated with change in BMLs over time. The strength of the association of physical activity with BML was very low and the results need to be interpreted cautiously. Most of these findings were based on cross-sectional data and therefore the causal pathways cannot be ascertained.

In conclusion, BMLs in young adults are associated with knee symptoms, a history of knee injury and other knee structural lesions. Moderate physical activity and HDL cholesterol are beneficially associated with BMLs, in contrast, vigorous physical activity is weakly but positively associated with medial tibofemoral BMLs. These suggest that BMLs are modifiable in young adults.

Chapter 9 - Summary and future directions

9.1 Summary

Osteoarthritis (OA) of the knee is a major, but poorly understood, public health problem. It is characterised by knee pain and structural abnormalities in the whole joint, eventually leading to total joint replacement in some cases. It is the most common joint disorder in the world. In Western populations, it is one of the most frequent causes of pain, loss of function and disability in adults [43]. With the ageing population and the rise in obesity rates, the social and economic burden associated with OA is increasing. In Australia, OA affects over 1.9 million people costing the health system $3.75 billion and the economy around $22 billion annually [46]. Over 85,000 knee and hip replacement procedures (the majority due to OA) were performed in Australian hospitals during 2012, each costing an average of $15,000–$31,900 [47]. Currently, the aetiology of OA is poorly understood, and there is no disease-modifying therapy for OA. Conventional treatment of OA is mostly palliative and costly. Clearly there is an urgent need to investigate the risk factors that cause the diseases from early life itself.

Identifying risk factors for OA is a major step towards preventing or treating OA. The major risk factors associated with the development of OA include age, female sex, obesity and joint injury, but there is no evidence to show how these factors in childhood are associated with the development of OA in later life. Specifically, there are no data relating childhood fitness or fatness to adult joint health. This thesis aimed to explore the childhood factors associated with adulthood knee joint health based on an existing cohort study that started from childhood. Knee magnetic resonance imaging (MRI) scans were obtained in this cohort. Knee joint health markers were quantified from these images using an image-processing software. The main aims of this thesis were to determine whether physical activity, physical fitness and fatness in childhood measured 25 years prior were associated with knee joint structure and symptoms in young adults aged 31-41 years. This thesis also aimed to explore the other correlates of knee structural and functional measures in these young adults.

The promotion of physical activity and fitness is a major public health initiative in western society. It is widely recommended to patients with knee and hip OA for improving their symptoms [143] although the effect of physical activity and fitness on the development and progression of OA is largely unclear. There is growing evidence that unlike in older population, physical activity in younger adults may be beneficially associated with knee structures [152, 153]. Based on this 25 years' cohort study, we found that a number of childhood physical performance measures, especially physical work

capacity at 170 beats per minute (PWC_{170}), were positively associated with knee tibial bone area and tibial cartilage volume in adulthood. The associations of childhood performance measures were independent of adulthood fitness measures. The associations with tibial cartilage volume appeared to be mediated in part by the tibial bone area. This suggests that physical activity in childhood can independently influence adult knee joint health possibly through adaptive mechanisms during growth. A healthy knee joint in young adult life would protect young people from developing knee OA in later life. Therefore, measures to improve physical fitness measures in children could be recommended to delay or prevent the development of knee OA.

Obesity is a major public health problem among adults as well as children and is associated with musculoskeletal conditions especially OA [16]. The knee joint is commonly affected by pain in both overweight paediatric [111] and adult populations [123]. However, there are only few studies suggesting an association between obesity and knee pain in general population and no studies have examined the effect of childhood overweight measures on adulthood knee pain, stiffness and dysfunction measured using standard scales. Therefore, we aimed to describe the associations between overweight measures in childhood and knee pain, stiffness and dysfunction among young adults 25 years later. We used age and sex specific cut off points for defining overweight in childhood [158] and used Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) scale to assess knee symptoms in adulthood. We found that childhood overweight measures were significantly associated with adulthood knee mechanical joint pain, stiffness and dysfunction among males, and these associations were independent of adult overweight measures. The change in overweight status from childhood to adulthood was also associated with knee pain, with subjects who were overweight in both childhood and adult life having the greatest prevalence and risk of knee pain. Subjects who were overweight in childhood and then of normal weight in adulthood also had a greater prevalence of knee pain than did subjects who were normal in childhood and then became overweight in adulthood. These findings suggests that childhood overweight may be more important than or at least as important as adult overweight and can lead to knee symptoms in later life. This research reveals that childhood overweight is an independent risk factor for the development of knee pain in young adults.

These findings, i.e., a protective effect of childhood physical performance measures and detrimental effect of childhood overweight measures on adult knee joint health, can be translated to improve awareness about maintaining good fitness and normal weight in childhood for a healthy knee joint in adulthood.

Higher knee cartilage volume (independent of bone size) in healthy young adults may protect against the development of OA in later life, and this would be similar to 'peak' bone mass protecting the development of osteoporosis in later life. Therefore, identifying all factors (including the adulthood factors in addition to childhood) associated with the 'peak' cartilage volume in young adults are of high priority. However, factors associated with the knee cartilage volume in a younger population are insufficiently explored. We aimed to describe the associations between body composition, hormonal and inflammatory factors measured five years prior and tibial cartilage volume in young adults. There is a well-known sex difference in knee cartilage volume (after considering body size difference) from childhood to late adulthood. We also aimed to confirm this sex difference in young adults and to explore if body composition and inflammatory factors explained the sex difference in tibial cartilage volume. We found that lean mass and sex hormone binding globulin were beneficially, and obesity measures and fibrinogen were detrimentally associated with tibial cartilage volume. Males had greater tibial cartilage volume than females and the sex difference in cartilage volume was largely explained by variations in body composition and fibrinogen levels. This study showed that body composition, sex hormones and fibrinogen correlated with knee cartilage volume in young adult life, suggesting the potential for intervention to increase cartilage volume: interventions aiming to regulate these factors may modify cartilage volume and protect the knee joint against OA in later life.

Physical activity has been recommended to patients with knee OA for improving their symptoms. However, there is very limited evidence for the association of physical activity and physical performance with knee structural measures such as tibial cartilage volume in young adults. It is possible that the effects of physical activity on knee cartilage volume in younger adults are different from those in older adults. We aimed to describe the associations between physical activity and physical performance measured five years prior and tibial cartilage volume and bone area in young adults. We found that physical activity and physical performance measures were beneficially associated with tibial cartilage volume whereas only physical performance was associated with tibial bone area. The association of performance measures with tibial cartilage volume was partially mediated by bone area. While tibial bone area is affected only by physical performance, tibial cartilage volume can be influenced by both physical activity and performance in younger adults. This suggests that the environmental factors such as physical activity can be beneficially associated with cartilage volume and can be considered as an intervention to improve cartilage volume.

Subchondral bone is rich in blood and nerve supply. There is emerging evidence to suggest that changes in bone precede cartilage damage, so that bone rather than cartilage may be the site initiating the significant pathophysiological events in OA [222]. Subchondral bone marrow lesions (BMLs) play a key role in the pathogenesis of OA [223] and are associated with pain [29] and other structural abnormalities. Most of these studies were in the older population, and little is known about BMLs of the knee joint in younger adults. We aimed to describe the prevalence and correlates of BMLs in younger adults. We found that BMLs in young adults were associated with knee pain and other knee structural abnormalities such as cartilage defects and meniscal lesions. Moderate physical activity and higher high-density lipoprotein cholesterol were protective, in contrast, vigorous physical activity is weakly but positively associated with BMLs, suggesting that BMLs are modifiable in young adults.

A strength of this thesis was the use of 25-year prospective data with physical activity and performance measures at baseline and tibial cartilage volume/bone area after 25 years. This thesis has several potential limitations. The response rate was low with only 43% of the persons invited to participate having MRI performed. Reassuringly, there were no significant differences in age, sex, BMI, and knee injury between those with and without MRI scans, or between subjects included in this study and the remainder of the original cohort, which suggests there was not major selection bias introduced. We did not measure knee malalignment in this study and could not account for the influence of alignment on the association between physical activity, physical performance, obesity and knee joint health. We had MRI measurement at one-time point at adulthood and did not have MRI measurements in childhood or at the time of physical activity or performance measurements. However, we have previously reported that the loss of cartilage volume mainly starts at the age of 40 [183, 206].

In conclusion, this series of related analyses of a childhood follow-up study provides considerable insight into the role of environmental factors such as optimal physical activity for better knee joint health in adulthood. Additionally, higher fitness and lower fatness in childhood and adulthood are beneficially associated with the better function and structural integrity of knee joint in young adults. Recommendations for the future directions are provided in the following section.

9.2 Future directions

This thesis has presented several novel findings from a unique 25-year follow-up study. The highlight is that childhood physical performance measures especially cardiorespiratory fitness was associated with adulthood knee cartilage volume. Similarly, adulthood physical performance measures including cardiorespiratory fitness were positively associated with knee cartilage volume. The association of childhood fitness with adulthood knee cartilage volume was independent of the adult attained fitness levels suggesting that improving physical fitness in any age is a possible intervention to attain higher cartilage volume. Aerobic exercise programmes have been recommended by the current guidelines for the treatment of OA in improving pain and function [243]. This is based on extensive studies comparing the efficacy of different exercise interventions on symptoms and function in patients with knee OA [244, 245]. However, no studies have focussed on the effect of aerobic exercise on knee structures including cartilage volume. The results from this thesis provide the preliminary data to support the need for a clinical trial with the focus on effects of exercise on structural measures of the knee.

In addition to cardiorespiratory fitness, body composition measures such as lean mass were beneficially associated with tibial cartilage volume. Fat mass and fibrinogen levels (an indicator of systemic inflammation) were detrimentally associated with tibial cartilage volume. Males had greater tibial cartilage volume than females in this young adult cohort of 31-41 years. One of the possible explanation for a lower prevalence of OA in males compared to females is that males have higher cartilage volume throughout their lives. This thesis suggested that the sex difference in cartilage volume was largely explained by variations in body composition and fibrinogen. Current guidelines for OA management recommend strengthening exercise, in particular, progressive resistance training (PRT), for pain relief and functional improvement [246]. In healthy older adults, multi-modal exercise programmes involving PRT and neuromuscular exercise containing functional weight-bearing activities can increase muscle mass, strength, and reduce fat mass and inflammatory mediators, all of which have been found to be associated with knee joint health in young adults from this thesis. However, it is unknown whether PRT can alter the structural components of the knee joint and further studies focusing on these structural outcomes are warranted.

The association of physical activity with knee structures is controversial. This thesis found that physical activity and physical performance measures assessed five years prior were beneficially associated with tibial cartilage volume. The association of physical

activity with tibial cartilage volume was independent of fitness measures suggesting that the environmental factors such as physical activity can be beneficially associated with cartilage volume independent of the genetic component acquired through fitness. Physical activity has been recommended for the management of OA in adults based on their ability to improve symptoms and function in OA. However, there are no studies that directly assessed the effect of physical activity on knee structures based on a randomised control trial. The preliminary data from this thesis support a beneficial effect of all types of physical activity including vigorous, moderate and total physical activity on cartilage volume. However, Chapter 8 of this thesis found that only moderate physical activity was beneficially associated with BML prevalence, and vigorous physical activity was detrimentally associated with BMLs. These findings from this thesis warrant further studies to identify the optimal level of physical activity for maintaining knee joint health.

One of the major limitations of this thesis is that we had only cartilage volume measurement at one-time point at adulthood. Following up this cohort for another MRI scan and quantifying the cartilage volume will allow us to calculate the cartilage volume loss over time in these subjects. Cartilage volume loss is known to be the best predictor of knee OA incidence and progression and can even predict total knee joint replacement. Further follow-up of this study will allow us to explore the childhood and adulthood predictors of cartilage volume loss, bone area change and progressions of BML, cartilage defect and meniscal pathology over time.

In conclusion, data from a childhood follow-up study indicate that physical performance measures in childhood and adulthood and physical activity in adulthood are associated with knee joint health measures. Conversely, childhood overweight measures are associated with increased knee pain in adulthood. Fatness measures in adulthood were negatively associated with knee cartilage volume indicating that obesity can play a significant role in the development of OA in later life. This thesis also supports a role for bone changes in the early stages of OA as evident from associations of BMLs with knee symptoms and other structural measures in this young population-based cohort. Future work should consider physical activity based treatments that change body composition and modify disease progression of knee OA.

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Appendices

Appendix 1 – CDAH Knee Cartilage study invitation letter

1 March 2008
«Title» «TFName» «TSurname»
«Address»
«State» «postcode»

Dear «TFName»

RE: **The CDAH Knee Cartilage Study**

We are writing to invite you to take part in a study, which follows from the CDAH study you participated in during 2004 to 2006. This study, known as the **CDAH Knee Cartilage Study,** is being conducted by a team of researchers led by Dr Changhai Ding at the Menzies Research Institute in Hobart and involving researchers from centres in Melbourne and Sydney. The study has been funded by the National Health & Medical Research Council.

The **CDAH Knee Cartilage Study** aims to determine whether childhood physical activity, fitness and body weight influence knee structure, knee pain and the risk of osteoarthritis of the knee in adulthood 23 years later. Osteoarthritis is characterised by a gradual loss of cartilage. It is the most common joint disorder in the world and causes pain and disability to those affected. The detailed information collected in the CDAH study in childhood and adulthood makes it one of the few studies in the world with the capacity to answer important questions about early-life causes of osteoarthritis. Further information about the study can be found in the enclosed Study Information Sheet.

Participation in the study involves a short telephone interview and an appointment for an MRI and X-ray scan at a nearby hospital as detailed below. If you agree to participate in this study, we would like you to read carefully through the enclosed Information Sheet, then complete the consent form, and return it to us using the reply-paid envelope as soon as you can. If you do not wish to participate, please indicate that on the form and we will not contact you again about the CDAH knee cartilage study again. If we do not hear from you, you may receive a reminder letter or follow-up telephone call.

After we receive your consent form, we will call you for a short telephone interview to update information about your lifestyle (occupation, smoking, physical activity) that may have changed over the last two years. We will also ask about your injury history (childhood and adulthood), any knee pain or stiffness you experience, and your physical function.

At the time of the telephone interview we will make an appointment for you (a coordinator in Sydney or Melbourne will also contact with you) to have a free knee MRI and X-ray

scan at the Alfred Hospital (residents of Melbourne) or the Royal North Shore Hospital (residents of Sydney). Your weight will also be measured at the hospital. Your individual results will be available to you if you require and, at a later date, a summary of the study's overall findings.

Participation in the study is entirely voluntary and you may withdraw from the study at any time. All information provided for the study will remain confidential and no individual will be identifiable in reports or presentations that arise from the study.

If you have any questions about the study, please contact the project officer XXX, on telephone number xxx.

Thank you again for your contribution to the CDAH study thus far and we hope you will accept our invitation to participate in the CDAH Knee Cartilage Study.

Yours sincerely,

Associate Professor A Venn, Deputy Director
Dr C Ding, Senior Research Fellow
Professor G Jones, Head, Musculoskeletal Unit

Appendix 2 – CDAH Knee Cartilage study consent form

OFFICE USE ONLY

Participant ID ☐☐☐☐☐

Consent Form Return Date

☐☐/☐☐/☐☐

The CDAH Knee Cartilage Study
CONSENT FORM

Chief Investigators – Dr C Ding
Professor G Jones
Associate Professor A Venn
Professor F Cicuttini
Professor T Dwyer
Associate Professor L March

1. I have read and understood the 'Information Sheet' for this study.
2. The purpose of the study, the expected length of time the examination will take, and an indication of any discomfort which may be expected, have been explained to me.
3. I understand that the study involves a telephone interview, wearing a pedometer for 7 days, weight measurement, MRI and X-Ray scans.
4. I agree to have knee MRI and X-ray measurements. I understand that this involves an amount of radiation exposure that is unlikely to be associated with increased risk of disease and is less than the current National Health & Medical Research Council guidelines for exposure to radiation in volunteers.
5. I understand that the MRI procedure may cause claustrophobia in someone in the first time but is otherwise safe.
6. I have been informed that the results of the study may not be of any direct benefit to my medical management.
7. Any questions that I have asked have been answered to my satisfaction.
8. I understand that all research data will be treated as confidential and no identifying information about me will be released.
9. I agree that research data gathered for the study may be published provided that I cannot be identified as a subject.
10. I understand that some of the results of my assessments may be given to me if I wish.
11. I agree to participate in this investigation and understand that I may withdraw at any time without prejudice.
12. I understand that the study has been approved by the Tasmanian Health & Medical Human Research Ethics Committee.

I consent to being part of the CDAH-Knee Cartilage Research Study

Name of Participant

Signature Date

_____

I *do not* wish to be part of the CDAH-Knee Cartilage Research Study

Name of Participant

Signature Date

Appendix 3 –CDAH Knee Cartilage study phone questionnaire

CDAH-Knee Cartilage Study

PHONE QUESTIONNAIRE

Personal Details:

Name:

First Name*

Middle Name(

Last Name:

Home address:

Post Code

Postal Address:

(if different)

Post Code

Telephone numbers:

Home: Work:

Mobile: **Email Address:**

Your Date of Birth: ☐☐ / ☐☐ / ☐☐☐☐

1. **(Females only)** Are you currently pregnant? Yes☐ **(**Exclude from the study**)**

No ☐

2. How tall are you? ☐☐☐ cm OR ☐☐ ft ☐☐ in

3. How much do you weigh? ☐☐☐ kg OR ☐☐ st ☐☐ lb

4. Which of the following describes your current employment status? You can pick more than one.

Working full-time ☐
Working part-time ☐
Not working (but not retired) ☐
Home duties ☐
Full-time student ☐
Part-time student ☐
Retired ☐
Permanently unable to work / ill ☐
Other ☐ (please specify)

_____

5. Which of the following best describes the occupation you had for the longest period?

Manager or administrator ..... ☐1

Professional ..... ☐2
(e.g. engineer, doctor, teacher, nurse, police, technical officer)

Tradesperson ..... ☐3
(e.g. carpenter, electrician, plumber, mechanic etc.)

Clerk ..... ☐4
(e.g. typist, receptionist, data processor, book keeper, etc.)

Salesperson or personal service worker ..... ☐5
(e.g. sales rep., teller, insurance rep., real estate rep.)

Plant or machine operator, or driver ..... ☐6
(e.g. taxi driver, bus driver)

Farmer ..... ☐7

Labourer or related worker ..... ☐8
(e.g. trade assistant, factory hand, agricultural labourer, construction, mining)

Member of armed forces ..... ☐9

Other, please state_____ .... ☐10

6. Had you had a knee injury requiring non-weight bearing treatment more than 24 hours or surgery during childhood?

Yes☐ No☐

If 'YES', what type of injury?

6a)_____

6b)_____

6c)_____

7. Have you had a knee injury requiring non-weight bearing treatment more than 24 hours or surgery in your adult life?

Yes☐ No☐

If 'YES', what type of injury?

6a)_____

6b)_____

6c)_____

8. Had you had any knee surgery during your childhood?

Yes☐ No☐

If 'YES', what type of surgery?

6a)_____

6b)_____

6c)_____

9. Have you had any knee surgery in your adult life?

Yes☐ No☐

If 'YES', what type of injury?

6a)_____

6b)_____

6c)_____

10. Have you had changed your smoking status since YOUR LAST INTERVIEW for this study?

Yes ☐ **(Answer Q.11-13 or Q.14)** No ☐ **SKIP TO: Q.15**

11. If you started smoking, when did you start smoking daily? ☐☐ Years of Age **OR** ☐☐☐☐ (Year)

12. If you started smoking, how often do you smoke cigarettes, cigars, pipes or any other tobacco products?

Daily ☐

At least weekly (but not daily) ☐

Less often than weekly ☐

Not at all ☐

13. If you started smoking, how many cigarettes, pipes or cigars do you smoke daily?

1-5 ☐

6-15 ☐

16-25 ☐

26-35 ☐

36-45 ☐

>45 ☐

14. If you stopped smoking, when did you finally stop smoking daily? ☐☐ Years of Age

OR ☐☐☐☐ (Year)

(Short Last 7 Days Telephone IPAQ)

READ: I am going to ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

READ: Now, think about all the *vigorous* activities which take *hard physical effort* that you did in the last 7 days. Vigorous activities make you breathe much harder than normal and may include heavy lifting, digging, aerobics, or fast bicycling. Think only about those physical activities that you did for at least 10 minutes at a time.

15. During the **last 7 days**, on how many days did you do **vigorous** physical activities?

_____ Days per week

☐ Don't Know/Not Sure

☐ Refused

[**Interviewer clarification**: Think only about those physical activities that you do for at least 10 minutes at a time.]

[**Interviewer note**: If respondent answers zero, refuses or does not know, skip to Question 16]

16. How much time did you usually spend doing **vigorous** physical activities on one of those days?

__ __ Hours per day

__ __ __ Minutes per day

☐ Don't Know/Not Sure

☐ Refused

[**Interviewer clarification**: Think only about those physical activities you do for at least 10 minutes at a time.]

[**Interviewer probe**: An average time for one of the days on which you do vigorous activity is being sought. If the respondent can't answer because the pattern of time spent varies widely from day to day, ask: "How much time in total would you spend **over the last 7 days** doing vigorous physical activities?"

__ __ Hours per week

__ __ __ Minutes per week

☐ Don't Know/Not Sure

☐ Refused

READ: Now think about activities which take *moderate physical effort* that you did in the last 7 days. Moderate physical activities make you breathe somewhat harder than normal and may include carrying light loads, bicycling at a regular pace, or doubles tennis. Do not include walking. Again, think about only those physical activities that you did for at least 10 minutes at a time.

17**.** During the **last 7 days**, on how many days did you do **moderate** physical activities?

_____ Days per week

☐ Don't Know/Not Sure

☐ Refused

[**Interviewer clarification**: Think only about those physical activities that you do for at least 10 minutes at a time]

[**Interviewer Note**: *If respondent answers zero*, refuses or does not know, skip to Question 18]

18. How much time did you usually spend doing **moderate** physical activities on one of those days?

__ __ Hours per day

__ __ __ Minutes per day

☐ Don't Know/Not Sure

☐ Refused

[**Interviewer clarification**: Think only about those physical activities that you do for at least 10 minutes at a time.]

[**Interviewer probe**: An average time for one of the days on which you do moderate activity is being sought. If the respondent can't answer because the pattern of time spent varies widely from day to day, or includes time spent in multiple jobs, ask: "What is the total amount of time you spent over the **last 7 days** doing moderate physical activities?"

__ __ Hours per week

__ __ __ Minutes per week

☐ Don't Know/Not Sure

☐ Refused

READ: Now think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you might do solely for recreation, sport, exercise, or leisure.

19. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ Days per week

☐ Don't Know/Not Sure

☐ Refused

[**Interviewer clarification**: Think only about the walking that you do for at least 10 minutes at a time.]

[**Interviewer Note**: *If respondent answers zero*, refuses or does not know, skip to Question 20]

20. How much time did you usually spend **walking** on one of those days?

__ __ Hours per day

__ __ __ Minutes per day

☐ Don't Know/Not Sure

☐ Refused

[**Interviewer probe**: An average time for one of the days on which you walk is being sought. If the respondent can't answer because the pattern of time spent varies widely from day to day, ask: “What is the total amount of time you spent walking over **the last 7 days**?”

__ __ Hours per week

__ __ __ Minutes per week

☐ Don't Know/Not Sure

☐ Refused

READ: Now think about the time you spent sitting on week days during the last 7 days. Include time spent at work, at home, while doing course work, and during leisure time. This may include time spent sitting at a desk, visiting friends, reading or sitting or lying down to watch television.

21. During the last 7 days, how much time did you usually spend ***sitting*** on a **week day**?

__ __ Hours per weekday

__ __ __ Minutes per weekday

☐ Don't Know/Not Sure

☐ Refused

[**Interviewer clarification**: Include time spent lying down (awake) as well as sitting]

[**Interviewer probe**: An average time per day spent sitting is being sought. If the respondent can't answer because the pattern of time spent varies widely from day to day, ask: "What is the total amount of time you spent *sitting* last **Wednesday**?"

__ __ Hours on Wednesday

__ __ __ Minutes on Wednesday

☐ Don't Know/Not Sure

☐ Refused

Standard Interview Script for SF-12v2 Health Survey
(4-week recall)

Script for interview administration:

READ: This next question is about your health *now***.**
Please try to answer as accurately as you can.

22. In general, would you say your health is **(read response choices)**
(Circle one number)

Excellent……1
Very good……..2
Good……..3
Fair…….4
Or Poor?……5

READ: Now I'm going to read a list of activities that you might do during a typical **day. As I read each item, please tell me if your health now limits you a lot, limits you a little, or does not limit you at all in these activities.**

23a. … moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf. Does your health now limit you a lot, limit you a little, or not limit you at all? **(Read response choices)**

(If respondent says s/he does not do activity, probe: *Is that because of your health?)*

(Circle one number)

Yes, limited a lot……1
Yes, limited a little……..2
Or, No, not limited at all?……..3

23b. … climbing several flights of stairs. Does your health now limit you a lot, limit you a little, or not limit you at all? **(Read response choices)**

(If respondent says s/he does not do activity, probe: *Is that because of your health?)*

(Circle one number)

Yes, limited a lot……1
Yes, limited a little……..2
Or, No, not limited at all?……..3

READ: The following two questions ask you about your physical health and your daily activities.

24a. During the past four weeks, how much of the time have you accomplished less than you would like as a result of your physical health? **(Read response choices)**

(Circle one number)

All of the time……1
Most of the time……2
Some of the time……..3
A little of the time……..4
Or None of the time?……5

24b. During the past four weeks, how much of the time were you limited in the kind of work or other regular daily activities you do as a result of your physical health? **(Read response choices)**

(Circle one number)

All of the time……1
Most of the time……2
Some of the time……..3
A little of the time……..4
Or None of the time?……5

Rate the following today for KNEES:

Example

Example of no pain ■0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

Example of severe pain ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ■9

25. Referring to your knees only, how much **PAIN** do you experience when

None

Severe

a. Walking on a flat surface ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

b. Going Up and down stairs ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

c. At night while in bed ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

d. Sitting or lying ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

e. Standing upright ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

26. Referring to your knees only, how much **STIFFNESS** do you experience

None Severe

a. After first awakening ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

b. Later in the day ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

27. Referring to your knees how much **FUNCTIONAL DEFICIT** do you experience when

None Severe

a. Descending stairs ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

b. Ascending stairs ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

c. Rising from bed ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

d. Rising from sitting ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

e. Putting on socks ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

f. Taking off socks ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

g. Bending to the floor ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

h. Lying in bed ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

i. Walking on flat ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

j. Getting in/out of bath ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

k. Standing ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

l. Sitting ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

m. Getting in/out of the car ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

n. Getting on/off toilet ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

o. Heavy domestic chores ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

p. Light domestic chores ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9

q. Shopping ☐0 ☐1 ☐2 ☐3 ☐4 ☐5 ☐6 ☐7 ☐8 ☐9