Arthritis Care & Research
DOI 10.1002/acr.22715

Body composition, hormonal and inflammatory factors are associated with tibial cartilage volume in young adults and contribute to the sex difference in cartilage volume

Running title: Correlates of tibial cartilage volume in young adults.

Key words: Young adults, body composition, sex hormones, inflammatory, sex difference, tibial cartilage volume

[1] Benny Antony, PhD student

[1] Alison Venn, PhD

[2] Flavia Cicuttini, PhD

[3] Lyn March, PhD

[1] Leigh Blizzard, PhD

[4] Terence Dwyer, PhD

[3] Marita Cross, PhD

[1] Graeme Jones, MD

[1,2] Changhai Ding, MD

[1]Menzies Institute for Medical Research, University of Tasmania, Hobart, Australia; [2]Department of Epidemiology and Preventive Medicine, Monash University, Melbourne, Australia; [3]Institute of Bone and Joint Research, University of Sydney, Sydney, Australia; [4]Murdoch Childrens Research Institute, Melbourne, Australia

Funding: National Health and Medical Research Council of Australia

Corresponding author and address for reprints: Professor Changhai Ding, Menzies Institute for Medical Research, Private Bag 23, Hobart, Tasmania 7000,

Received: Dec 20, 2014; Revised: Aug 11, 2015; Accepted: Aug 18, 2015

Australia; Tel: 61-3-62267730, Fax: 61-3-62267704,

e-mail: changhai.ding@utas.edu.au

Conflict of interest statement: No conflict of interest to declare.

Word Count: 4149

Abstract

Objective:

To describe the associations between body composition, hormonal and inflammatory factors measured 5 years prior and tibial cartilage volume in young adults and to explore if these factors contribute to the sex difference in tibial cartilage volume.

Methods:

Subjects broadly representative of the young adult Australian population (n=328, aged 31-41 years, female 47.3%) were selected. They underwent T1-weighted fat-suppressed magnetic resonance imaging (MRI) of their knees. Tibial cartilage volume was measured from MRI. Sex hormone binding globulin (SHGB) and testosterone in a subset of females and C-reactive protein (CRP) and fibrinogen in both sexes were measured 5 years prior. Body mass index (BMI), fat mass and lean mass were calculated from height, weight and skinfolds.

Results:

In multivariable analyses, correlates of tibial cartilage volume included lean body mass (β: 26.4mm^3, 95%CI: 13.6,39.1), fat mass (β: -11.8mm^3, 95%CI: -22.2,-1.4), and fibrinogen (β: -146.4mm^3, 95%CI: -276.4,-16.4) but not BMI, testosterone and CRP. In females, SHBG was positively associated with tibial cartilage volume (β: 0.67mm^3, 95%CI: 0.14,1.20) and free androgen index was negatively associated with lateral tibial cartilage volume (β: -0.04mm^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.

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.

Significance

- Lean body mass and sex hormone binding globulin are beneficially associated with tibial cartilage volume in young adults.
- Fat mass and inflammatory markers such as fibrinogen are detrimentally associated with tibial cartilage volume in young adults.
- The sex difference in cartilage volume is contributed largely by variations in body composition and fibrinogen levels.
- Factors including body composition, sex hormones and fibrinogen correlate with knee cartilage volume in young adult life.

Introduction

Osteoarthritis (OA) is the most common joint disorder and the most common cause of disability in adults around the world, characterized 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 visualization of whole joint structures and the ability to manipulate the MR images to generate quantitative data on cartilage such as cartilage volume (1).

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 (2, 3), and in lateral compartment than medial compartment (3). Given the fact that knee OA is 1.5 times more common in women than in men (4) and 4 times more common in the medial compared with the lateral compartment, low cartilage volume may be a risk factor for knee OA (5).

The associations of body composition (6-8), inflammatory (9, 10) and hormonal factors (11) 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 (8). 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 (6). 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 (12). We have reported that in older adults, circulating levels of IL-6 and TNF-alpha were associated with knee cartilage loss (10). Furthermore, C reactive protein (CRP), a systemic marker of inflammation, was found to be negatively associated with knee cartilage volume in midlife women (9). 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 (13). There were very few studies that examined the association between sex hormones and MRI- assessed knee structures (14), 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 contribute to sex difference in tibial cartilage volume in young adults.

Materials and methods

Subjects

The Childhood Determinants of Adult Health (CDAH) Knee Study was conducted during 2008-2010. It was a follow-up study on a sub-sample (n=328, mean age 35, range 31-42) of participants in the CDAH study (n=2410, mean age 31, range 26-36). CDAH study was conducted during the period of 2004 to 2006 and included Australia-wide subjects aged between 26 to 31 years. The CDAH participants (n=764)

residing in metropolitan Melbourne and Sydney were contacted by mails and invited to participate in the CDAH Knee Cartilage study. Subjects who agreed to participate (n=529, response percentage 69%) were assessed for their eligibility. Exclusion criteria included being pregnant, having had diseases that may affect knee cartilage such as rheumatoid arthritis, or having a contraindication for MRI. Eighty subjects were excluded, and the remaining 449 subjects were asked to complete a short computer-assisted telephone interview with knee injury recorded in childhood and adulthood in response to the question "Have you had a knee injury requiring non-weight bearing treatment for more than 24 hours or surgery?" They were requested to have a 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. Two MR images were not readable and were excluded. 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, and all participants provided written informed consent.

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 0.1cm.

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 skinfolds 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 skinfolds according to published equations for adults (15) and lean body mass (LBM) and fat mass was calculated using weight (kg) (LBM = weight – ((fat% × weight)/100)).

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 (16). Free androgen index (FAI), the active testosterone in the blood, was calculated as: testosterone (nmol/L) / SHBG (nmol/L).

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).

MRI measurements

Participants had an MRI scan of their dominant knee in the CDAH Knee Cartilage study. MRI scans were obtained from two hospitals, which used the same type of 1.5T scanner (General Electric Medical Systems, Milwaukee, WI, USA). Knees were imaged in the sagittal plane on a whole body magnetic resonance unit with use of a commercial transmit-receive extremity coil. The following image sequence was used: a T1-weighted fat saturation 3D 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).

This imaging sequence has enabled us to reconstruct the cartilage and to measure the volume (2). Individual plates of tibial cartilage volume (medial and lateral) were isolated from the total volume 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 (17). The

coefficients of variation (CVs) for cartilage volume measures were 2.1-2.6% (18). We used total tibial cartilage volume (calculated as the sum of medial and lateral tibial cartilage volume) for analysis because the predictor measures were of systemic (not local) in nature and should have similar effect on both medial and lateral compartment.

The bone area of medial and lateral tibial plateau was measured manually using the T1-weighted 3D MRI. Images were reformatted to axial plane and the average area of the three MR images closest to tibial cartilage in the axial plane was measured (18-20). The CVs for these measures were 2.2-2.6% (18). Total tibial bone area was calculated as the sum of medial and lateral area.

Ten volunteers had MRI scans performed at both hospitals' MRI machines to determine the variation between machines. 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 another machine.

Statistical analyses

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).

Results

Characteristics of the subjects

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 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.

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 2); however, fat mass (Figure 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 2). In contrast, lean body mass (Figure 1b) and percentage lean body mass were positively associated

with tibial cartilage volume (Table 2). There were no significant interactions between sex and obesity measures (except for WHR, p=0.062) on tibial cartilage volume.

Hormonal factors and tibial cartilage volume

SHBG measured in 50% of female subjects (n=78) 5 years prior was positively associated with total tibial cartilage volume in univariable and multivariable analysis (Table 3). Conversely, testosterone in 50% of females (n=78) showed a negative trend, but was not significantly, associated with tibial cartilage volume (Table 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 3). All these association remained after further adjustment for fat mass.

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 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 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 of borderline significance (16% reduction in the effect size) after further adjustment for fat mass (p=0.064).

Sex difference in tibial cartilage volume

As shown in Table 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 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 5). The magnitude of sex difference in tibial cartilage volume remained largely unchanged after adjustment for waist hip ratio or CRP (8% reduction), but decreased by 37% after adjustment for fibrinogen and the association became non significant (Table 5).

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, waist hip ratio and fibrinogen were negatively associated with tibial cartilage volume, and fat mass or fibrinogen explained significant part (20% and 37%, respectively) 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 (21) and there was no significant difference in the loss of tibial cartilage volume between men and women before the age of 40 (22). Subjects in our study were of mean age 36 and predominantly healthy and are expected to remain at the "peak" cartilage volume of their lifetime. Therefore, we speculated that, similar as peak bone mass that is a strong predictor of future risk of osteoporosis in older people (23), "peak" cartilage volume in younger adults 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 (18, 24), cartilage defects (24) and symptoms (25). 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 (20); 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 (26) or healthy knees (27, 28) although a few found that BMI was negatively associated with cartilage volume (5). We reported that BMI was associated with knee cartilage loss only in people within the highest tertile of baseline knee cartilage volume (7). 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 waist hip ratio 5 years prior were negatively associated with tibial cartilage volume in these young adults. Similar results were observed longitudinally in healthy (29) and older adults (8) 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 (6) and muscle strength (30) 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 (31). 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 (6).

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; (5) however, serum free testosterone was associated with an increased rate of cartilage loss in its follow-up study (32). 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 (33). SHBG is more stable compared to sex hormones and there is an emerging recognition that low levels of SHBG are associated with increased proinflammatory states associated with insulin resistance, and low SHBG can be an indicator of low-grade inflammation associated with the metabolic syndrome in obese individuals (34). 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 (11). A potential physiological explanation of our findings is that SHBG is behaving as a surrogate marker for global estrogen exposure (11) 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 (14). This is supported by a clinical study, which reported that post-menopausal women using long-term estrogen replacement therapy (ERT) had more knee cartilage than controls (35).

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 (9). We have reported that inflammatory cytokines including IL-6 and TNF alpha are associated with the cartilage loss;(10, 36) 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 (37). Fibrinogen was reduced after the weight loss in patients with knee OA although it didn't correlate with the symptom reduction (38). Similarly, fibrinogen levels were reduced after supplementation of calcium fructoborate in knee OA patients (39). 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. There was no gender difference 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 of 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 skinfolds 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. However, there were no significant differences in terms of age, duration of follow-up, BMI and tibial cartilage volume between female subjects who underwent hormone measurements and those did not. Fourth, we did not have MRI scans at the time of obesity or blood measures and cartilage volume was measured 5 year after the predictor measures. It is possible that the amount of cartilage volume at the time of predictor measures was different; however, we are unlikely to see significant cartilage loss over time in this young population as significant knee cartilage volume loss starts at the age of 40 (21). Similarly, we did not measure the predictor measures such as fibrinogen, CRP and sex hormones at the time of MRI. Fifth, the participants in our study were of 31-41 years old and we speculated them to remain the "peak" cartilage volume of their life time which might protect them from developing knee OA (17); however, there is no direct evidence so far to show when the maximum cartilage volume of a person is attained and there is no longitudinal evidence to suggest that the "peak" cartilage volume will protect from developing knee OA in later life. Last, the MRI sequence we used in this study was unable to differentiate cartilage swelling from normal cartilage volume; however, participants in our group were from general young adult population and mostly healthy.

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

Acknowledgements

Special thanks go to the National Health and Medical Research Council of Australia that funded this study and to the subjects who made this study possible. The roles of Liz O'Loughlin and Judy Hankin and in collecting the data, Marita Dalton in managing the database, Rob Warren in processing the MR images and Petr Otahal in statistical analyses are gratefully acknowledged. C. Ding is a recipient of ARC Future Fellowship, G. Jones is a recipient of a NHMRC Practitioner Fellowship, A. Venn is a recipient of a NHMRC Senior Research Fellowship, and L. Blizzard is a recipient of a NHMRC Career Development Fellowship.

Author contribution

Antony had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study design: Ding, Jones, Venn, Cicuttini, Dwyer and March; Acquisition of data: Ding, Jones, Venn, Cicuttini, Dwyer, Cross, and March; Analysis and interpretation of data: Antony, Ding, Venn, Blizzard and Jones; Manuscript preparation: Antony, Ding, Venn, Blizzard, Cicuttini, Dwyer, March, Cross, and Jones; Statistical analysis: Antony, Ding, Blizzard and Jones.

Conflict of interest disclosure:

All authors have completed the Competing Interest form (available on request from the corresponding author) and declare: no support from any organisation for the submitted work; no financial relationships with any organisations that might have an interest in the submitted work in the previous three years; no other relationships or activities that could appear to have influenced the submitted work.

Financial disclosure:

National Health and Medical Research Council of Australia funded this study and

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

References

1. Ding C, Zhang Y, Hunter D. Use of imaging techniques to predict progression in osteoarthritis. Curr Opin Rheumatol. 2013;25:127-35.

2. Cicuttini F, Forbes A, Morris K, Darling S, Bailey M, Stuckey S. Gender differences in knee cartilage volume as measured by magnetic resonance imaging. Osteoarthritis Cartilage. 1999;7:265-71.

3. Jones G, Glisson M, Hynes K, Cicuttini F. Sex and site differences in cartilage development: A possible explanation for variations in knee osteoarthritis in later life. Arthritis & Rheumatism. 2000;43:2543-9.

4. Srikanth VK, Fryer JL, Zhai G, Winzenberg TM, Hosmer D, Jones G. A meta-analysis of sex differences prevalence, incidence and severity of osteoarthritis. Osteoarthritis Cartilage. 2005;13:769-81.

5. Cicuttini FM, Wluka A, Bailey M, O'Sullivan R, Poon C, Yeung S, et al. Factors affecting knee cartilage volume in healthy men. Rheumatology (Oxford). 2003;42:258-62.

6. Berry PA, Wluka AE, Davies-Tuck ML, Wang Y, Strauss BJ, Dixon JB, et al. The relationship between body composition and structural changes at the knee. Rheumatology (Oxford). 2010;49:2362-9.

7. 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. J Rheumatol. 2011;38:1973-80.

8. Ding C, Stannus O, Cicuttini F, Antony B, Jones G. Body fat is associated with increased and lean mass with decreased knee cartilage loss in older adults: A prospective cohort study. Int J Obes (Lond). 2012.

9. Hanna FS, Bell RJ, Cicuttini FM, Davison SL, Wluka AE, Davis SR. High sensitivity c-reactive protein is associated with lower tibial cartilage volume but not lower patella cartilage volume in healthy women at mid-life. Arthritis Res Ther. 2008;10:R27.

10. Stannus O, Jones G, Cicuttini F, Parameswaran V, Quinn S, Burgess J, et al. Circulating levels of il-6 and tnf-alpha are associated with knee radiographic osteoarthritis and knee cartilage loss in older adults. Osteoarthritis Cartilage. 2010;18:1441-7.

11. Hanna FS, Bell RJ, Cicuttini FM, Davison SL, Wluka AE, Davis SR. The relationship between endogenous testosterone, preandrogens, and sex hormone binding globulin and knee joint structure in women at midlife. Semin Arthritis Rheum. 2007;37:56-62.

12. Jin X, Beguerie JR, Zhang W, Blizzard L, Otahal P, Jones G, et al. Circulating c reactive protein in osteoarthritis: A systematic review and meta-analysis. Ann Rheum Dis. 2013.

13. de Klerk BM, Schiphof D, Groeneveld FP, Koes BW, van Osch GJ, van Meurs JB, et al. No clear association between female hormonal aspects and osteoarthritis of the hand, hip and knee: A systematic review. Rheumatology (Oxford). 2009;48:1160-5.

14. Linn S, Murtaugh B, Casey E. Role of sex hormones in the development of osteoarthritis. PM R. 2012;4:S169-73.

15. Durnin JV, Rahaman MM. The assessment of the amount of fat in the human body from measurements of skinfold thickness. 1967. Br J Nutr. 2003;89:147-55.

16. Wei S, Schmidt MD, Dwyer T, Norman RJ, Venn AJ. Obesity and menstrual irregularity: Associations with shbg, testosterone, and insulin. Obesity (Silver Spring). 2009;17:1070-6.

17. Antony B, Jones G, Venn A, Cicuttini F, March L, Blizzard L, et al. Childhood physical performance measures predict adulthood knee cartilage volume and bone area: A 25-year cohort study. Arthritis Care Res (Hoboken). 2015.

18. Jones G, Ding C, Scott F, Glisson M, Cicuttini F. Early radiographic osteoarthritis is associated with substantial changes in cartilage volume and tibial bone surface area in both males and females. Osteoarthritis Cartilage. 2004;12:169-74.

19. Cicuttini FM, Wluka AE, Stuckey SL. Tibial and femoral cartilage changes in knee osteoarthritis. Ann Rheum Dis. 2001;60:977-80.

20. Ding C, Cicuttini F, Scott F, Cooley H, Boon C, Jones G. Natural history of knee cartilage defects and factors affecting change. Arch Intern Med. 2006;166:651-8.

21. Ding C, Jones G, Wluka AE, Cicuttini F. What can we learn about osteoarthritis by studying a healthy person against a person with early onset of disease? Curr Opin Rheumatol. 2010;22:520-7.

22. Ding C, Cicuttini F, Blizzard L, Scott F, Jones G. A longitudinal study of the effect of sex and age on rate of change in knee cartilage volume in adults. Rheumatology (Oxford). 2007;46:273-9.

23. Winsloe C, Earl S, Dennison EM, Cooper C, Harvey NC. Early life factors in the pathogenesis of osteoporosis. Curr Osteoporos Rep. 2009;7:140-4.

24. Ding C, Garnero P, Cicuttini F, Scott F, Cooley H, Jones G. Knee cartilage defects: Association with early radiographic osteoarthritis, decreased cartilage

volume, increased joint surface area and type ii collagen breakdown. Osteoarthritis Cartilage. 2005;13:198-205.

25. Wluka AE, Wolfe R, Stuckey S, Cicuttini FM. How does tibial cartilage volume relate to symptoms in subjects with knee osteoarthritis? Ann Rheum Dis. 2004;63:264-8.

26. Wluka AE, Stuckey S, Snaddon J, Cicuttini FM. The determinants of change in tibial cartilage volume in osteoarthritic knees. Arthritis Rheum. 2002;46:2065-72.

27. Wluka AE, Wolfe R, Davis SR, Stuckey S, Cicuttini FM. Tibial cartilage volume change in healthy postmenopausal women: A longitudinal study. Ann Rheum Dis. 2004;63:444-9.

28. Ding C, Cicuttini F, Scott F, Cooley H, Jones G. Knee structural alteration and bmi: A cross-sectional study. Obes Res. 2005;13:350-61.

29. Wang Y, Wluka AE, English DR, Teichtahl AJ, Giles GG, O'Sullivan R, et al. Body composition and knee cartilage properties in healthy, community-based adults. Ann Rheum Dis. 2007;66:1244-8.

30. Foley S, Ding C, Cicuttini F, Jones G. Physical activity and knee structural change: A longitudinal study using mri. Med Sci Sports Exerc. 2007;39:426-34.

31. Racunica TL, Teichtahl AJ, Wang Y, Wluka AE, English DR, Giles GG, et al. Effect of physical activity on articular knee joint structures in community-based adults. Arthritis Rheum. 2007;57:1261-8.

32. Hanna F, Ebeling PR, Wang Y, O'Sullivan R, Davis S, Wluka AE, et al. Factors influencing longitudinal change in knee cartilage volume measured from magnetic resonance imaging in healthy men. Ann Rheum Dis. 2005;64:1038-42.

33. Davis SR, Burger HG. The rationale for physiological testosterone replacement in women. Baillieres Clin Endocrinol Metab. 1998;12:391-405.

34. Hammond GL, Wu TS, Simard M. Evolving utility of sex hormone-binding globulin measurements in clinical medicine. Curr Opin Endocrinol Diabetes Obes. 2012;19:183-9.

35. Wluka AE, Davis SR, Bailey M, Stuckey SL, Cicuttini FM. Users of oestrogen replacement therapy have more knee cartilage than non-users. Ann Rheum Dis. 2001;60:332-6.

36. Stannus OP, Cao Y, Antony B, Blizzard L, Cicuttini F, Jones G, et al. Cross-sectional and longitudinal associations between circulating leptin and knee cartilage thickness in older adults. Ann Rheum Dis. 2013.

37. Papageorgiou N, Tousoulis D, Siasos G, Stefanadis C. Is fibrinogen a marker of inflammation in coronary artery disease? Hellenic J Cardiol. 2010;51:1-9.

38. Richette P, Poitou C, Garnero P, Vicaut E, Bouillot JL, Lacorte JM, et al. Benefits of massive weight loss on symptoms, systemic inflammation and cartilage turnover in obese patients with knee osteoarthritis. Ann Rheum Dis. 2011;70:139-44.

39. Scorei R, Mitrut P, Petrisor I, Scorei I. A double-blind, placebo-controlled pilot study to evaluate the effect of calcium fructoborate on systemic inflammation and dyslipidemia markers for middle-aged people with primary osteoarthritis. Biol Trace Elem Res. 2011;144:253-63.

Table 1. Baseline characteristics of the participants

	Females n=155	Males n=173	P-value
CDAH Knee Cartilage Study			
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
CDAH Study (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
CDAH: childhood determinants of adult health study
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.

Table 2. Association between obesity measures assessed 5 years prior in CDAH study 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

CDAH: childhood determinants of adult health study

BMI: body mass index

LBM: lean body mass

Table 3. Association of sex hormones measured 5 years prior in CDAH study 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 (n=78)
*Adjusted for age, sex, BMI, duration of follow-up, injury and tibial bone size
No interactions between sex and predictors on cartilage volume
CDAH: childhood determinants of adult health study
SHBG: sex hormone-binding globulin
Free androgen index was calculated as: testosterone/SHBG

Table 4. Association of inflammatory markers measured 5 years prior in CDAH study 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
CDAH: childhood determinants of adult health study
CRP: c reactive protein

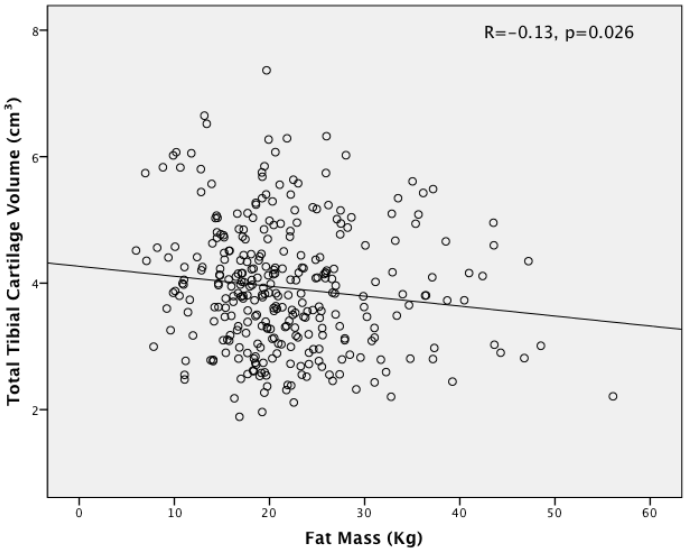
Table 5. Sex difference in knee cartilage volume: mediating effects of body composition and inflammatory factors

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

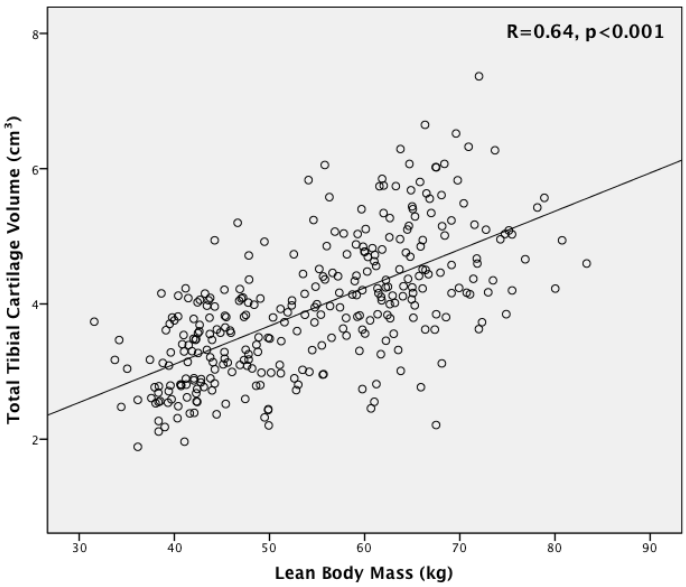
*Adjusted for age, duration of follow-up, height, weight, injury and tibial bone size
Model 1: further adjustment for lean mass (instead of weight)
Model 2: further adjustment for fat mass (instead of weight)
Model 3: further adjustment for lean mass and fat mass (instead of weight)
Model 4: further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

Figure legends

Figure 1.
Association between body fat mass (a) and lean body mass (b) and tibial cartilage volume



(a)



(b)

Figure 1