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The Effect of Exercise and Ageing on Morphology and Biomarkers of Knee Articular Cartilage in Healthy Humans

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The Effect of Exercise and Ageing on Morphology and Biomarkers of Knee Articular Cartilage in Healthy Humans

By

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List of publications

Roberts, H. M., Moore, J. P., Griffith-McGeever, C. L., Fortes, M. B., & Thom, J. M. (2016). The effect of vigorous running and cycling on serum COMP, lubricin, and femoral cartilage thickness: a pilot study. *European Journal of Applied Physiology*. 116, 1467-1477.

Conference proceedings

Roberts, H. M., Moore, J. P., Griffith-McGeever, C. L., Fortes, M. B., & Thom, J. M. (2015). The effect of vigorous running and cycling on novel markers of knee joint function. Presented in a thematic poster presentation session at the ACSM Annual Meeting, San Diego, CA., USA (2015).

Thesis summary

The knee joint is a complex weight bearing joint that has a high incidence of degenerative change and knee OA. Consequently, there is a need to develop a greater understanding of factors that may affect knee articular cartilage and influence long term health of the knee joint. The overall aim of this thesis was to specifically investigate the effect of ageing and physical activity on several serum biomarkers that have been found to have a different profile among individuals with degenerative knee conditions such as OA. These biomarkers included: serum COMP, which is understood to be a marker of cartilage degeneration or metabolism; serum HA, which is also understood to be both a marker of cartilage degeneration and synovial inflammation; serum lubricin, which is understood to be a marker of joint lubrication; and finally, femoral cartilage thickness assessed by ultrasound (US), which provides a morphological measurement of the articular cartilage. Firstly, in Chapter 4, the feasibility of measuring femoral cartilage thickness using US was determined. This study demonstrated high intra-tester reliability, measurement precision, and revealed that femoral cartilage thickness has a large variability in healthy individuals across a range of ages. In a subsequent cross-sectional study (Chapter 5), results indicated that ageing does not inevitably result in degenerative change of articular cartilage in healthy males. Moreover, physical activity was not associated with any adverse changes to joint markers, and instead, was associated with greater lateral condyle cartilage thickness in this cohort. Chapters 6 and 7 specifically explored acute joint loading. Acute exercise increased serum lubricin and COMP with no change in serum HA. The increase in serum COMP and lubricin suggests an increase in cartilage metabolism and joint lubrication, respectively. However, somewhat surprisingly this response did not differ between weight bearing and non-weight bearing exercise. The follow up study compared aerobic exercise with resistance training. Despite substantial differences in the loading magnitude and frequency between lower body resistance exercise and walking exercise, both exercise modalities resulted in a similar response in serum biomarkers. There was also no difference between the exercise response in both healthy male and female individuals. Overall, this research has provided new evidence to suggest that both healthy ageing and physical activity are not associated with adverse changes to the articular cartilage. Moreover, while acute exercise typically results in a significant increase in both serum COMP and lubricin, neither exercise modality nor gender appears to alter the response to acute loading.

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List of abbreviations

ACL	Anterior Cruciate Ligament
ACSM	American College of Sports Medicine
ANCOVA	Analysis of Covariance
ANOVA	Analysis of Variance
BMI	Body Mass Index
CI	Confidence Interval
COMP	Cartilage Oligomeric Matrix Protein
CV	Coefficient of Variation
ELISA	Enzyme-Linked Immunosorbent Assay
HA	Hyaluronan
HRmax	Maximum Heart Rate
ICC	Intraclass Correlation Coefficient
IPAQ	International Physical Activity Questionnaire
K/L	Kellgren-Lawrence
MAX	Maximum
MET	Metabolic Equivalent
mRNA	Messenger Ribonucleic Acid
MPH	Miles Per Hour
MRI	Magnetic Resonance Imaging
OA	Osteoarthritis
PRG-4	Proteoglygan 4
RA	Rheumatoid Arthritis
RM	Repetition Maximum
RPE	Rate of Perceived Exertion
rpm	Revolutions per minute
SD	Standard Deviation
SEM	Standard Error of the Measurement
SRD	Smallest Real Difference
SSHES	School of Sport Health and Exercise Science
US	Ultrasound
VO_2	Oxygen Uptake
WOMAC	Western Ontario and McMaster Universities Osteoarthritis Index

1 CHAPTER 1. General introduction

Health care professionals advocate physical activity and the use of exercise across the lifespan to promote health and to prevent disease. However, some of the exercises used to promote good health place direct stress on joints. As a weight-bearing joint, the knee is particularly susceptible to knee stress and is a primary site of injury, pain and degenerative conditions such as osteoarthritis (OA) (Bollen, 2000; Peat et al. 2006). Knee injuries have previously been reported at an incidence rate of 2.29 knee injuries per 1,000 population (Gage et al. 2012). Moreover, in relation to knee OA, a UK based population study with a 5-year follow up reported an incidence rate of knee OA (KL $\geq$ 2) as 2.3% per year (Hart et al. 1999). Similarly, the Framingham Osteoarthritis Study, which utilised an approximately 8-year follow-up, reported an incidence rate of knee OA (KL $\geq$ 2) of 1.4% per year in Caucasian men and 2.2% per year in Caucasian women (Felson et al. 1995). Although physical activity is correctly used as both a preventative and therapeutic strategy of knee injury and knee OA, there remain many concerns regarding the effect of physical activity and its effect on the knee joint (Muthuri et al. 2011; Ratzlaff et al. 2012). This is particularly true for occupational activities, as well as weight bearing activities such as distance running, which have previously been associated with an increased risk of knee OA (Cymet and Sinkov, 2006; Felson et al. 1991). Until further research can determine more conclusively if physical activity *per se* is safe and positive for the knee joint, then the general population will not be convinced that this is the case. In addition, ageing, obesity, female gender and knee joint injury are common risk factors of knee OA (Anderson and Loeser, 2010; Grotle et al. 2008; Muthuri et al. 2011; Paradowski et al. 2006) that may also influence the relationship between physical activity and the knee joint, and warrant further investigation. Hence, there is a clear need to research further the effect of exercise, as well the impact of additional risk factors on the status of the knee joint.

Currently, the assessment of the knee joint primarily relies on methods to quantitatively assess the knee joint structures. Radiography remains the first tool for the assessment of clinical OA and is considered the gold standard in clinical and epidemiological setting (Wang et al. 2012). Magnetic resonance imaging (MRI) has also been successfully used to assess knee OA (Hunter et al. 2011), cartilage morphology (Eckstein et al. 2006), cartilage composition (Matzat et al. 2013), as well as cartilage deformation in response to exercise

(Eckstein et al. 1999). However, a promising alternative imaging method for the evaluation of the knee is ultrasonography. This tool has been successfully used to measure femoral cartilage thickness in several clinical populations (Abraham et al. 2011; Akkaya et al. 2013a; Kara et al. 2013; Kaya et al. 2012). Biochemical indices, including, structural molecules or fragments related to the knee joint, which can be assessed in blood, urine or synovial fluid are also promising measures to monitor the knee joint (Abramson and Krasnokutsky, 2006). Both ultrasonography and biochemical markers are techniques that may assist existing established outcome measures in developing a greater understanding of the role of ageing and exercise on intra-articular knee health.

A key area of research for the current thesis was to establish both the normal range of femoral cartilage thickness measurements assessed by ultrasonography, as well as the normal values of several novel serum biochemical indices of joint damage. To date, there remains limited literature exploring variables including age, sex, weight, and previous joint injury with femoral cartilage thickness assessed by ultrasound (US), or with novel serum biochemical indices of joint damage. Using these potential early indicators of joint damage, this thesis aims to investigate possible associations with previously identified risk factors of OA across healthy individuals of a range of ages and physical activity levels. Moreover, although physical activity is an established preventative and therapeutic strategy for several major health conditions, including diabetes (Sigal et al. 2007), obesity (Strasser, 2013), and cardiovascular disease (Lavie et al. 2009), the effect on the knee joint is not fully understood. The impact of physical activity on the knee joint, including the risk of OA is likely to be dependent on the type of physical activity, as well as the frequency and intensity of activity. Factors such as age, sex, weight, and previous injury may also influence the relationships between physical activity and knee joint, and thus warrant further investigation. Therefore, this thesis will investigate the effect physical activity on several knee joint markers.

2 CHAPTER 2. Review of literature

2.1 Introduction to cartilage morphology

Articular cartilage is a low-friction, wear-resistant tissue that covers the subchondral bone of synovial joints and is designed to resist tensile and compressive forces (Pearle et al. 2005). Although a highly specialised tissue with unique mechanical properties, articular cartilage is primarily an avascular and aneural tissue that has low metabolic activity (Hunziker et al. 2002). Articular cartilage consists of a dense extra-cellular matrix together with a small population of cells, known as chondrocytes. Chondrocytes make up about 2% of the total volume of articular cartilage (Alford, 2005) and their metabolism is responsible for developing, maintaining and repairing the extracellular matrix (Goldring, 2012). The extracellular matrix consists of two components: a tissue fluid and a framework of collagens, proteoglycans and non-collagenous proteins (Buckwalter and Mankin, 1997). It is the interaction between the fluid and extracellular matrix components that provide cartilage with its viscoelastic and mechanical properties for efficient load distribution (Mow and Guo, 2002). Aggrecan and type II collagen are understood to be the most abundant proteins within the articular cartilage extra-cellular matrix (Roughley and Mort, 2014) and are linked together by several non-collagenous proteins. Aggrecan, attached to hyuloranan via a link protein, forms a large proteoglycan aggregate, which together with a network of collagen fibrils, form the solid phase of the extracellular matrix and provide the biomechanical properties of articular cartilage (Fox et al. 2009).

Articular cartilage can be divided into four zones, all of which have different functional and structural properties, these include: the superficial zone, the middle zone, the deep zone, and the zone of calcified cartilage (Figure 2-1). The superficial tangential zone, is the outermost layer, and is composed of a layer of densely packed collagen fibrils arranged parallel to the surface (Mow and Guo, 2002) and has a low concentration of proteoglycan (Muir et al. 1970). This zone makes up approximately 10-20% of the cartilage thickness and is understood to have a high number of flattened chondrocytes (Muir et al. 1970), which have lubricating and protective functions (Waller et al. 2013). In contrast, the middle zone makes up 40-60% of the cartilage and has thicker collagen fibrils which are more rounded and randomly organized compared to superficial zone (Clarke, 1971). There is also a rise of proteoglycan content in the middle zone, which helps provide a higher compressive modulus

compared to the superficial tangential zone (Maroudas, 1979). The deep zone consists of bundles of collagen fibrils oriented perpendicular to the articular surface and makes up approximately 30-40% of the cartilage. The deep zone also has the highest proteoglycan content, and the lowest water concentration (Maroudas, 1979). Typically, chondrocytes are orientated in a columnar fashion positioned parallel to the collagen fibres (Clarke, 1971). The calcified zone rests directly on the subchondral bone and is identified by the tide-mark which separates the deep zone from the calcified cartilage. The calcified layer helps secure the cartilage to bone and functions by anchoring the collagen fibrils of the deep zone to subchondral bone (Fox et al. 2009). Although the cell population is limited, the calcified zones is vascularised and innervated from the subchondral bone (Clark, 1990).

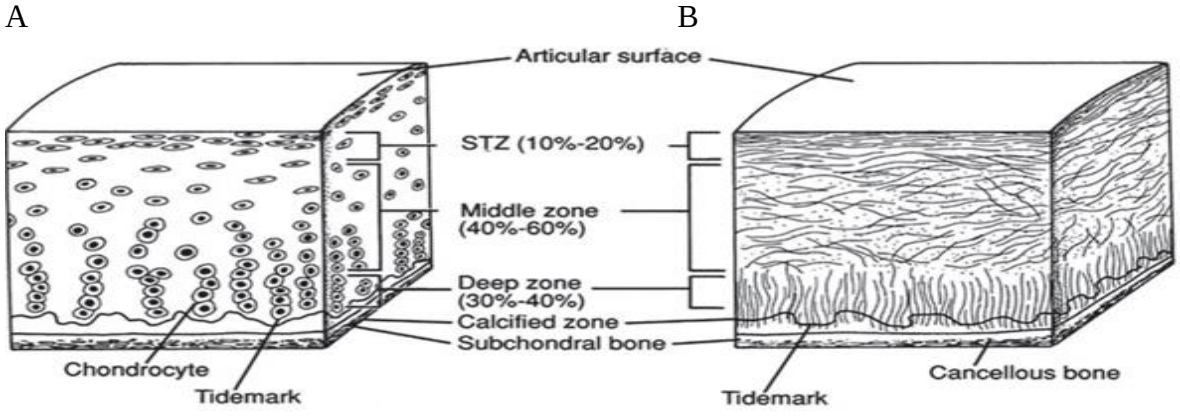


Figure 2-1 A schematic diagram demonstrating A) chondrocyte organisation and B) collagen fibre organisation in healthy articular cartilage (STZ = superficial tangential zone) [From: Buckwalter et al. (1994)]

2.2 Function and dysfunction of articular cartilage

Articular cartilage is understood to have no function other than maintaining mechanical competence (Eckstein et al. 2006). Healthy human articular cartilage functions by providing a smooth surface, capable of transferring large loads evenly across the subchondral bone (Eckstein et al. 2001; Mow et al. 1984). The mechanical properties of the articular cartilage tissue i.e. the response to loading and unloading of the joint are dependent on the interaction of the fluid and solid phases. Much of the fluid contained within the interstitial intrafibrillar space, which is created by collagen-proteoglycan solid phase, remains in place due to the negative charge of proteoglycans and the subsequent affinity to water (Maroudas, 1976). Furthermore, proteoglycans, entangled and compacted, offer low levels of permeability,

which together with the low permeability of articular cartilage surface, are important in determining the movement of fluid within the matrix (Pearle et al. 2005). Hence, these properties provide the compressive stiffness and the viscoelastic properties of articular cartilage observed in response to loading and unloading (Eckstein et al. 1999).

In contrast, articular cartilage indicative of OA, i.e. a model of articular cartilage dysfunction, is associated with softening, fibrillation and erosions, and the eventual loss of cartilage; subchondral remodelling in the form of sclerosis and osteophyte formation; swelling and inflammation of the synovium (Krasnokutsky et al. 2008). With OA, the initial changes to articular cartilage comprise of changes in composition, including the loss of proteoglycan content. There is an increase in the proteolytic degradation of proteoglycans resulting in smaller proteoglycan chains (Mankin et al. 1999). Alterations in proteoglycan structure can result in a more permeable solid matrix, leading to a reduction in the hydraulic pressure within the extracellular matrix and may consequently causes a reduction in compressive stiffness (Buckwalter and Mankin, 1997).

2.3 Factors associated with increased risk of knee OA

Knee OA is responsible for over half of all OA cases (Arthritis Research UK, 2013) and affects more than 6 million people in the UK (Peat et al. 2006). Knee OA is a major cause of disability and pain, with consequences including, immobility, knee arthroplasty, and poor quality of life (Woolf and Pfleger, 2003). Due to progress in molecular biology there has been a move away from the traditional 'wear and tear' paradigm of OA (Pearle et al. 2005). It is now understood that multiple risk factors and pathophysiological processes contribute to OA (Figure 2.2), initiating the process of injury and promoting biochemical processes that contribute to alterations to cartilage, subchondral bone and synovium (Krasnokutsky et al. 2008). Ageing is consistently associated with increased risk of OA (Felson et al. 1987) and is often considered to be the single biggest risk factor of knee OA (Anderson and Loeser, 2010). Several studies have also demonstrated that women have a higher prevalence of knee OA compared to men, i.e. a greater proportion of women demonstrate both radiographic OA (Felson et al. 1997) and symptomatic OA and pain compared to men (Felson et al. 1987; Paradowski et al. 2006). Moreover, the incidence rate of knee OA is higher in women, increasing dramatically around the time of menopause (Srikanth et al. 2005). This coincides with the menopause and may relate to an oestrogen reduction in females post menopause

(Sniekers et al. 2010). Moreover, genetic factors, likely in combination with other risk factors, are also understood to have a role development of knee OA (Spector et al. 1996a). While ageing, gender and genetics are non-modifiable risk factors, many factors that are associated with knee OA are modifiable. A primary modifiable risk factor for knee OA is obesity (Grotle et al. 2008; Murphy et al. 2008). Although obesity-associated increases in mechanical loading are likely to be a key factor in the increased incidence of OA of the knee, obesity also increases the inflammatory status of individuals, which may also increase the risk of OA (Berenbaum, 2013). Several studies have also demonstrated that knee joint injury, including anterior cruciate ligament (ACL) injury (Øiestad et al. 2009) as well as injury to the meniscus (Englund et al. 2009) increase the likelihood of future OA, possibly through an increase in knee joint instability. Joint malalignment has also been found to be an independent risk of future OA (Tanamas et al. 2009). Thus, joint stabilisation following injury and the correcting abnormal alignment may be key to optimising the long-term health of the knee joint and reducing the prevalence of OA. A further modifiable risk factor for the development of knee OA is physical activity. Occupational physical activity is often associated with OA, particularly in professions that involve repetitive loading through kneeling or squatting (Felson et al. 1991). Sport participation may also increase the risk of OA (Felson et al. 1987; Spector et al. 1996b). However, this is not a universal finding (Chakravarty et al. 2008; Hannan et al. 1993) and instead may relate to certain sports, elite athletes, or individuals with previous sport-related injuries (Bosomworth, 2009). Moreover, given that muscle dysfunction and quadriceps weakness have been associated with the development of knee OA (Shrier, 2004), exercise that maintains quadriceps function may instead be protective, enabling the redistribution of the excessive forces (Segal et al. 2010; Segal et al. 2012).

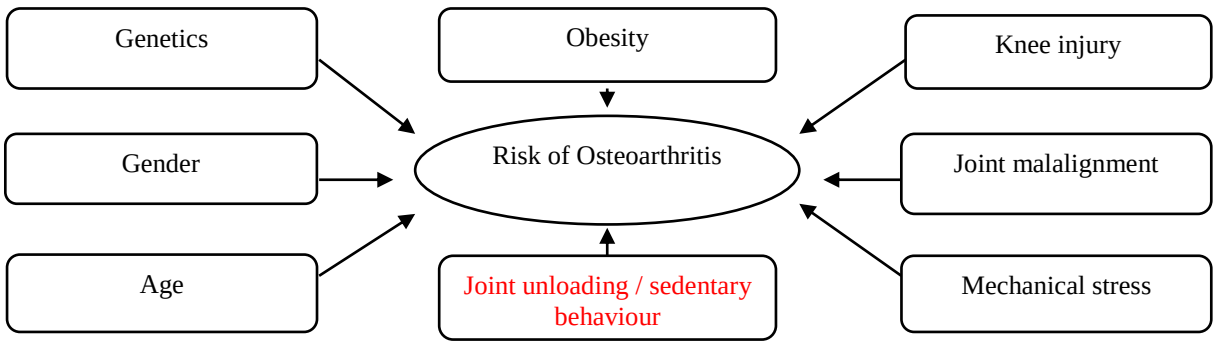


Figure 2-2 Risks of knee osteoarthritis

2.4 Methodological approaches to assess articular cartilage morphology

The ability to quantitatively assess the status of articular cartilage is important given that joint diseases such as OA are characterised by cartilage abnormalities, erosion, and loss of hyaline articular cartilage (Amin et al. 2005). The quantitative assessment of cartilage allows the diagnosis of degenerative change, OA disease progression, and the evaluation of therapeutic strategies. Moreover, assessing cartilage morphology may also provide an opportunity to determine the effect of mechanical joint loading and the study of functional adaptation of cartilage to mechanical loading (Stammberger et al. 1999). There are currently multiple imaging modalities that are used for the study of articular cartilage in humans, such as radiography, MRI and ultrasonography.

Radiography is the first line diagnostic tool for the assessment of knee OA in a clinical setting and is currently considered the gold standard technique for assessing joint damage (Wang et al. 2012). Radiography of the knee allows the evaluation of joint space narrowing, which is used as a surrogate measure of cartilage morphology. Joint space narrowing was previously assessed using manual techniques, however, recent improvements in precision and reproducibility have largely been related to the introduction of automated assessment. Radiography, together with the Kellgren-Lawrence (K/L) grading system, is the most widely used and accepted method for the clinical diagnosis of OA. Furthermore, currently radiography detected joint space narrowing remains the only accepted imaging endpoint in clinical trials (Conaghan et al. 2011). However, radiography has several shortcomings including an inability to directly visualise the articular cartilage and a discordance between radiographic OA and both knee pain and function (Bedson and Croft, 2008). Several studies have also demonstrated that joint space narrowing is insensitive to early cartilage loss when compared to MRI (Amin et al. 2005; Jones et al. 2004). MRI is a technique that provides a non-invasive, three-dimensional assessment of the knee joint, whilst allowing the direct visualisation of articular cartilage. The recent development of dedicated MRI sequences has enabled excellent soft tissue contrast and for the quantitative measurement of cartilage morphology across multiple planes (Wang et al. 2012). Several studies have demonstrated good evidence of measurement validity, accuracy and reliability (Eckstein et al. 2006; Multanen et al. 2009; Raynauld, 2003). MRI has also been recommended as an alternative modality for the assessment of cartilage morphology in clinical trials (Conaghan et al. 2011). Despite several advantages compared to radiography, i.e. no exposure to harmful radiation,

MRI is expensive, time consuming, requires significant expertise, and is not as widely available for clinical management or research. However, methodological approaches to assess cartilage morphology and OA are not limited to radiography and MRI. Ultrasonography has also emerged as a promising tool to assess the knee joint.

2.4.1 The use of ultrasonography to assess cartilage thickness

High resolution ultrasonography is a simple, inexpensive and non-invasive method that enables knee articular cartilage to be assessed. Supra-patellar transverse and longitudinal imaging are both techniques that allow the directly visualisation of the femoral cartilage and have previously been used to quantitatively assess cartilage thickness (Yoon et al. 2008). Cartilage appears as a homogenous anechoic band comprised between a synovial space-cartilage interface and cartilage bone interface. This subsequently allows the measurement of cartilage thickness from one interface to the other. The lack of echoes and image sharpness of the synovial space-cartilage and cartilage bone interface is typical of healthy and undamaged cartilage, while in contrast, poorly defined, or complete lack of a synovial space-cartilage or cartilage bone interface is indicative of degenerative change and late OA, respectively.

The most frequently used technique to assess knee articular cartilage is a supra-patellar transverse US technique. This technique exposes the femoral cartilage and is performed with the participant lying in a supine position with the knee in maximal flexion and the US transducer placed transversely and superior to the patellar border (Naredo et al. 2009; Yoon et al. 2008). The assessment of femoral articular cartilage by supra-patellar transverse ultrasound has previously been shown to demonstrate both good validity and reliability in normal to moderately damaged joints (Abraham et al. 2011; Naredo et al. 2009). Several studies have successfully measured cartilage thickness using this technique in both healthy populations and several clinical cohorts, including knee OA, following partial meniscectomy, systemic lupus, and spinal cord injury (Abraham et al. 2011; Akkaya et al. 2013b; Kara et al. 2013; Kaya et al. 2012).

Compared to other modalities such as radiography and MRI, US is quick, reasonably inexpensive and has no contra-indications to its use (Iagnocco, 2010). US is also unique in that it provides real time imaging with immediate feedback. However, unfortunately, due to a narrow acoustic window, ultrasonography is largely limited to the femoral plate. This presents an obvious limitation compared to MRI, which not only offers the ability to assess

other cartilage plates but also the option to assess cartilage volume as opposed to thickness alone. Additionally, US is considered operator dependent, a factor that may influence the reliability considerably (Kane et al. 2004; Skou and Aalkjaer, 2013). Overall, US is an attractive technique to assess articular cartilage, however, further studies are warranted to determine the reliability of cartilage thickness measurements.

2.5 Cartilage morphology in health and disease

Research demonstrates that considerable inter-subject variability exists in the thickness of cartilage within the human knee. For example, in an MRI study of healthy male individuals (age 23 to 64 years) mean knee cartilage thickness has previously been shown to range from 1.57 to 2.76 mm (Eckstein et al. 2001). Mean cartilage thickness was greatest at the patella (2.76 mm) and thinnest at the medial and lateral femoral condyle (1.57 mm and 1.58 mm, respectively). The greatest maximal cartilage thickness reported was at the patella (7.75 mm) and was followed by femoral trochlea (6.71 mm) and lateral tibia (6.10 mm). This demonstrates surprisingly high variability in the distribution of cartilage within the knee joint. In a similar cross-sectional study using ultrasonography, Özçakar and colleagues (2014) explored mean femoral cartilage thickness in a large sample (n = 1438) of healthy male and female individuals aged between 25–40 years. As per Eckstein et al. (2001), overall femoral cartilage thickness was thickest at the intercondyle notch (2.30 mm) compared to the lateral (2.20 mm) and medial (2.20 mm) condyles. Although femoral intercondyle thickness appeared to be comparable to the thickness reported using MRI, thickness at the lateral and medial femoral condyles appeared to be greater in this sample.

Differences also exist in the knee articular cartilage dimensions of men and women (Faber et al. 2001). Women have been shown to have significantly reduced cartilage volume at the femur (-27%), medial tibia (-46.6%), and lateral tibia (-43.3%), as well as reduced cartilage thickness at the femoral trochlea (-2%) and medial tibial plate (-13.3%). Comparable reductions have also been demonstrated in femoral cartilage thickness of women when assessed by US (Özçakar et al. 2014). Differences in cartilage thickness between men and women have typically been much less pronounced when compared to cartilage volume. This is understood to be largely due to the greater articular surface area of males compared to female joints. Moreover, height and weight, which are both typically greater in men, have both been correlated with cartilage variables (Hudelmaier et al. 2003), suggesting that body

size may also be important in cartilage morphology (Ding et al. 2005). Sex differences in knee loading dynamics (Cicuttini et al. 1999) as well as differences in the sex hormone oestrogen may also be responsible for differences observed in cartilage morphology (Sniekers et al. 2010).

Studies have also demonstrated that cartilage thickness is reduced in several clinical populations, including OA and rheumatoid arthritis (RA). Medial cartilage thickness assessed by US has previously been found to be negatively associated with knee pain (Abraham et al. 2014). Moreover, cartilage volume loss has been associated with the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC) pain changes over a 24-month period in patients with knee OA (Raynauld et al. 2006). In contrast, Kazam et al. (2011) found no difference in cartilage thickness between patients with symptomatic and asymptomatic knee OA. The importance of reduced cartilage morphology on pain, may relate to the stage of loss, or the symptoms in their participants. For example, greater rates of cartilage loss have been found in painful knees than in pain-free knees after adjustment for radiographic disease stage (Eckstein et al. 2001). However, given that cartilage must dissipate stress and provide a frictionless surface during joint movement, it would seem logical that greater cartilage thickness would benefit positive joint health.

Considerable variability exists in the cartilage morphology of both healthy and clinical populations. Elucidating the determinant that regulates the distribution of articular cartilage tissue in knee joints is crucial to understanding the process of cartilage loss in degenerative knee joint disease. Cartilage loading has been shown to be key in the maintenance of normal cartilage morphology. Therefore, variables that influence the dynamic loading of the knee joint may be predictive of the cartilage distribution throughout the joint. Of interest in this thesis is the role of ageing and physical activity. These are two key variables that are associated with cartilage loading and may explain some of the variability observed in cartilage thickness.

2.6 The effect of ageing on cartilage morphology

With age, articular cartilage undergoes substantial physiologic, mechanical, biochemical, and functional changes that reduce its ability to overcome the effects of mechanical stress and injury (Luria and Chu, 2013). These alterations impair the remodelling and repair processes, which may subsequently increase the vulnerability of tissue to degenerative change (Martin and Buckwalter, 2002). Several studies using MRI have indicated that ageing is associated with surface fibrillation of articular cartilage (Arokoski et al. 2000; Pap et al. 1998; Young et al. 2006), an increased number of defects (Ding et al. 2005), and a reduction in cartilage volume (Ding et al. 2005; Hanna et al. 2005; Mosher et al. 2010) and thickness (Hudelmaier et al. 2001; Ding et al. 2005).

In a large sample of healthy individuals (aged between 26-61 years), Ding et al. (2005) demonstrated that age was negatively associated with knee cartilage thickness at all sites (b= - 0.013 to -0.035 mm/year). These negative associations remained significant after adjustment for sex, height, weight, case-control status, bone size at that site, and radiographic OA. In a separate study, knee cartilage thickness was also found to be thinner in asymptomatic older individuals (50-78 years) compared with younger asymptomatic individuals (20-30 years) (Hudelmaier et al. 2001). The greatest reduction in knee cartilage was found at the femoral plate, with a -21% reduction among elderly women and a -13% reduction among elderly men. This study indicates that with age, knee cartilage thickness is reduced, but that the level of reduction differs between sexes and between knee joint compartments. Typically, in healthy individuals, overall cartilage thickness has been shown to reduce by 0.3-0.5% annually (Hudelmaier et al. 2001). In contrast, in patients with radiographic OA, total tibiofemoral thickness has been shown to be reduced by 0.8-1.3% in the 1st year following diagnosis and by 0.7-0.8% in 2nd year (Eckstein et al. 2009). This indicates that the age-associated decline in individuals with radiographic OA is greater than healthy individuals, particularly in the early stages of diagnosed OA (Eckstein et al. 2009). Age has also been found to be negatively associated with femoral cartilage assessed by supra-patellar US (Özçakar et al. 2014). In this large study (n = 1438) of healthy volunteers (aged between 25–40 years), age was negatively associated with right medial condyle thickness in women, while in men, age was negatively associated with both right and left lateral condyle thickness, as well as both right and left intercondyle notch thickness. This is currently the only study that has explored age associated

femoral cartilage thickness using US. Further studies are required using US, particularly in samples with a greater age range.

2.7 The effect of physical activity and chronic exercise training on cartilage morphology

Physical activity is known to increase muscle mass and bone mineral density (Bemben and Bemben, 2011), while inactivity or microgravity conditions are associated with tissue atrophy (Williams et al. 2009). However, whether functional adaptation of the articular cartilage exists in adult human cartilage remains to be fully elucidated.

In humans, three-months of either endurance training or strength training (3 times / week) has previously shown to have no effect on cartilage morphology (thickness or volume) assessed by MRI in untrained women aged between 45–55 years (Cotofana et al. 2010). Similarly, six-months of marathon training consisting of between 25–60 km/week in men and women (mean age of 39.9 ± 3.8 years) demonstrated no significant change in cartilage thickness, except for a small decrease (1.7 ± 1.6%) at the lateral femoral condyle, which was comparable to MRI precision error (Hinterwimmer et al. 2013). Evidence suggests that although short term increases in knee joint loading are well tolerated and do not lead to clinically relevant losses in cartilage, no increases in cartilage morphology are observed.

Several cross-sectional studies have also used MRI to explore knee cartilage thickness between athletes and non-athletic or sedentary controls. For example, triathletes completing regular training (minimum of 10 hours per week over 3 years) demonstrated no difference in cartilage thickness compared with individuals who completed less than 1 hour of sport per week (Muhlbauer et al. 2000). Similarly, Gratzke et al. (2007) investigated the morphological characteristics of professional weight lifters and sprinters (aged between 19-35 years) to ascertain if cartilage thickness was sensitive to high magnitude mechanical loading. All athletes had at least 7 years training experience and were highly strength trained. Results demonstrated that weightlifters and sprinters had greater patellar cartilage thickness than non-athletic controls (+14% and +17% respectively), but that no significant difference was observed in any other joint surfaces.

In contrast, a further study using MRI demonstrated that marathon runners (with a mean training history of at least 5 years) have significantly greater femoral cartilage thickness compared to sedentary controls (Mosher et al. 2010). This study also demonstrated that the difference in cartilage thickness between marathon runners and sedentary controls was considerably greater in the older individuals (>45 years of age) at both the femoral and tibial cartilage plates. Therefore, exercise training appears to be particularly important for cartilage thickness in older individuals. Moreover, a large cross-sectional study (between 25-40 years), which used US to assess femoral cartilage thickness, demonstrated that men who regularly exercised had thicker cartilage than men who did not engage in exercise (Özçakar et al. 2014). Interestingly, there was no difference in femoral cartilage thickness between men who engage in 1-2 sessions of exercise per week and those who engaged in 3 or more exercise sessions per week.

To date research on the effect of exercise on cartilage morphology in adult humans is mixed. There is currently no clear evidence that cartilage thickness changes in response to either short-term exercise training (6 months or less), or longer, sustained periods of chronic mechanical loading. One explanation for this observation is that there is no advantage of increased cartilage thickness beyond a normal and healthy level. However, greater muscle strength and fitness, especially in women, has been shown to be protective against cartilage loss (Foley et al. 2007). Likewise, regular exercise training may prevent or delay an age-associated decline in cartilage thickness (Mosher et al. 2010). Further research is required, particularly relating to the type, intensity and overall exercise volume, on cartilage morphology.

2.8 The effect of acute exercise on articular cartilage morphology

The behaviour of cartilage in response to acute loading is known as deformational behaviour and is understood to be dependent upon its biochemical composition (Eckstein et al. 2005). Deformational behaviour provides an additional sensitive measure of degenerative change than morphological properties such as cartilage thickness and volume (Burstein et al. 2000). Patello-femoral and femoro-tibial cartilage deformation has largely been studied in relation to changes in cartilage volume; however, studies have also explored cartilage thickness deformation post exercise. The possibility of measuring cartilage deformation was first

studied in a small sample (n = 8) of healthy individuals using MRI (Eckstein et al. 1998). In this study, patellar cartilage volume was significantly decreased (mean: 6%; range 2.4 - 8.6%) post exercise i.e. 3-7 minutes following 50 deep knee bends. There was a small decrease in patellar cartilage throughout the entire joint surface. This was the first study to demonstrate measurable cartilage deformation in response to exercise. Following this initial investigation, MRI techniques have been further refined and knee cartilage deformation across the full knee joint has been investigated. For example, Kersting et al. (2005) explored the effect of a 1-hour training run on cartilage deformation across all cartilage plates. Results demonstrated that all cartilage bodies reduce in volume post exercise, with only the medial tibial cartilage demonstrating a non-significant decrease.

Several studies have also explored cartilage deformation following several different types of physical activity (e.g. Eckstein et al. 2005; Niehoff et al. 2011). In a cross-sectional MRI study of healthy volunteers, patellar cartilage volume was shown to be significantly reduced by 5.9% after knee bends, 5.0% after running, 4.7% after squatting, 4.5% after cycling, and 2.8% after normal walking (Eckstein et al. 2005). In the same study, the deformational behaviour of the femoro-tibial cartilage was also investigated following knee bends, static compression, and high impact loading. Unlike patellar cartilage the femoral cartilage remained largely unchanged across the different activities, while significant medial (-6.1%) and lateral (-7.2%) tibial cartilage deformation only occurred following the high impact jumps. Niehoff et al. (2011) compared cartilage thickness and volume deformation assessed by MRI, following 30 minutes of running (distance of 3.96 km) and drop landing (total of 100 drop jumps) in healthy adults. Running largely resulted in greater cartilage deformation compared to the drop jump intervention, although, the difference between interventions was only significant for lateral tibial volume and thickness. Overall, these results suggest that the deformational response to loading depends on the activity and that the cartilage plates appear to have different mechanical and biochemical properties.

A further MRI study investigating cartilage deformation provided evidence to suggest that the level of cartilage deformation may be load dependent (Kessler et al. 2006). The study by Kessler and colleagues assessed patella and tibia cartilage volume before and after 5, 10, and 20 km of running in male athletes. Mean patellar volume significantly decreased by 6.6%, 6.1%, and 8.1% following 5, 10, and 20 km respectively. Similarly, mean tibial volume also significantly decreased by 3.6%, 5.0%, and 6.1% for the 5, 10, and 20 km, respectively. A

later follow up study by Kessler and colleagues also revealed that despite a significant change in patella and tibia cartilage volume immediately following 20 km of running, the cartilage tissue volume had almost completely returned to pre-run levels following an hour of rest (Kessler et al. 2008). This data suggests that in trained runners' cartilage tissues can adapt to the loads associated with running (Kessler et al. 2008).

To determine whether exercise training alters the deformational behaviour to cartilage, Eckstein et al. (2005) used MRI to compare patellar cartilage deformation following knee bends in 7 professional weight lifters, 7 sprinters, and 14 untrained volunteers. In untrained volunteers, there was a reduction in patellar cartilage volume after knee bends of 4.1%. In trained participants, the reduction was similar for bobsleigh sprinters (3.9%) but slightly less in the weight lifters (2.9%). While all groups demonstrated significant cartilage deformation in response to exercise, there were no significant differences between the different training statuses. Currently no study has directly investigated whether endurance trained individuals demonstrate different deformational behaviour than non-trained individuals. However, in relation to endurance training, 30 minutes of running in healthy recreational runners results in a mean percentage deformation of 5.7% in the lateral tibial cartilage, 3.3% in the medial tibial cartilage, 4.0% in the lateral femoral cartilage, and 5.3% in the medial femoral cartilage (Boocock et al. 2009). In comparison, in highly trained endurance runners, 1 hour of running resulted in significant cartilage volume reduction of 3.1% in lateral tibial area but a non-significant decrease in medial tibial area, while there was a significant 2.1% reduction in femoral cartilage volume (Kersting et al. 2005). Therefore, despite an increase in running duration, the trained runners demonstrated reduced deformation, possibly a functional adaptation because of chronic exercise training. Further studies are warranted to ascertain whether this conclusion is valid.

2.9 Introduction to biomarkers

Biomarkers can be defined as objectively measured indicators of normal biological processes, pathogenic processes, or pharmacologic responses to therapeutic interventions (Atkinson et al. 2001). Biochemical markers in the blood, urine or synovial fluid offer an attractive alternative to traditional methods to assess OA and may aid early diagnosis, prognosis, and allow the response to therapeutic strategies to be assessed. These biomarkers include molecules or molecular fragments from the extracellular matrix or cellular metabolism of the

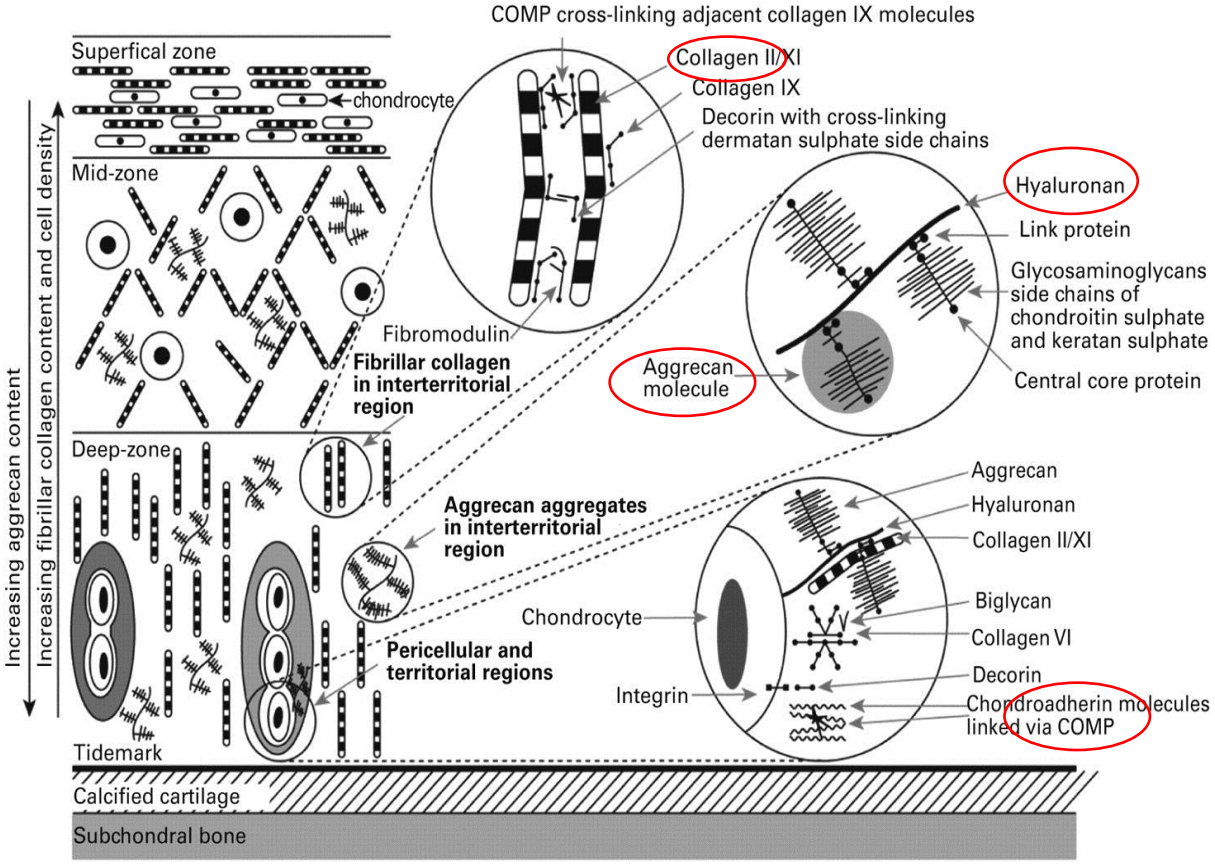
articular cartilage, subchondral bone and synovial issue. Several inflammatory biomarkers, including cytokines, chemokines as well as signalling molecules and growth factors may also be potential markers of OA.

The Osteoarthritis Biomarker Network is a classification scheme for biomarkers associated with OA and degenerative change that was recently proposed (Bauer et al. 2006). This new approach, known by the acronym BIPED, represents a system that is founded on establishing the relative merits of biomarkers by utilising the following five categories: **B**urden of disease, **I**nvestigative, **P**rognostic, **E**fficacy of intervention, and **D**iagnostic. Several recent reviews have documenting the relative merits of various OA biomarkers (Attur et al. 2013; Lafeber and Van Spil, 2013; Van Spil et al. 2010). Potential biomarker candidates for the present research were assessed on their performance in line with the BIPED criteria and whether biomarkers demonstrated strong validity and reproducibility. Studies specifically documenting the validity of serum biomarkers are limited. However, studies have demonstrated a correlation between serum COMP and radiographic severity (r = 0.370), which is considered the gold standard for the measurement of OA, as well as MRI score (r = 0.654) of knee OA (El-Arman et al. 2010). Serum HA has also been positively correlated (r = 0.410) with the severity of OA and the number of knee OA joints (r = 0.395) (Inoue et al. 2011), and the rate of joint space narrowing over 5 years (r = 0.404) (Sasaki et al. 2015). Both serum COMP and HA levels are known to vary within studies, with a considerable overlap often demonstrated between individuals with and without knee OA. It has been previously reported that serum COMP offers an excellent distinction between individuals with knee OA and controls (sensitivity: 98%, specificity: 98.0%) (Singh et al. 2015). Similarly, HA levels show an ability to discriminate between individuals with knee OA and controls (sensitivity: 95.0%, specificity: 90.0%), between mild and moderate cases (sensitivity: 87.6%, specificity: 86.0%) and between moderate and severe cases (sensitivity: 92.3 %, specificity: 93.1%). (Singh et al. 2015). Likewise, the use of COMP to predict RA has been shown to be high (r = 0.864) (Liu et al. 2016). Using the COMP cut-off of 21.51 ng/ml to rule out and rule in the presence of RA, the same study correctly classified 81.7% (i.e. sensitivity) of patients as having RA and correctly classified 88.2% (i.e. specificity) as not having RA was (Liu et al. 2016). Despite some promising results, currently used biomarkers remain insufficiently discriminating for the diagnosis or prognosis of degenerative change (Van Spil et al. 2010). Consequently, the assessment of the knee joint

may be enhanced when several serum biomarkers of joint metabolism are utilised to assess the joint.

2.9.1 Promising candidates for exploring the knee joint

To identify promising biomarkers, an analysis of peer-reviewed journals was undertaken prior to 31/12/2013. This review focused on structural molecules or fragments related to joint cartilage and the extracellular matrix that had been previously associated with knee joint OA and/or early knee degeneration. These biochemical markers are typically associated with joint tissue synthesis, degradation, or metabolism and can be assessed in body fluid such as synovial fluid, serum and urine. Several of the most investigated biomarkers were identified and presented in (Figure 2-3). These include biomarkers related to collagen metabolism, aggrecan metabolism, as well as non-collagenous proteins. An overview of the biomarkers, including key findings can be found in Table 2-1.



Biomarkers related to collagen metabolism

- C-terminal telopeptide of collagen type II (CTX-II)
- C-propeptide of type II procollagen (sCPII)
- C-telopeptide of type II collagen / Collagen synthesis (uCTX-II/sCPII)
- Collagen type II specific neoepitope (CIIM)
- N-propeptide of type IIA procollagen (PIIANP)

Biomarkers related to non-collagenous proteins

- Serum Cartilage Oligomeric Matrix Protein (sCOMP)
- Serum Hyaluronan (sHA)

Biomarkers related to aggrecan metabolism

- Core protein fragments (i.e. aggrecan ARGS)
- Serum Chondroitin Sulphate 846 (sCS846)
- Serum Keratan Sulphate (sKS)

Figure 2-3 Selected biomarkers that have previously been investigated for the evaluation of knee osteoarthritis, together with the possible sources of the biomarkers. Red circles = promising structural fragments that were reviewed for use within the present thesis; figure adapted from Pollard et al. (2008)

Table 2-1 A summary of selected structural molecules or fragments that have previously shown promise as biomarkers of collagen, aggrecan and non-collagenous protein metabolism

Biomarker	Marker of.	Commercially avail. ELISA for urine or serum	Summary
		Biomarkers related to collagen metabolism	
C-telopeptide of type II collagen (uCTX-II)	Collagen degradation	Yes	56% higher in patients with knee OA (Dam et al. 2009); elevated levels are associated with a 3.1% increased yearly cartilage loss (Dam et al. 2009); Patients with uCTX-II levels above the median for the 5-year average were associated with a higher risk of progression with a relative risk (95% CI) of 3.4 (1.2–9.4) (Sharif et al. 2007). Unchanged following 30 min of acute exercise (Helmark et al. 2012). Limited acute exercise studies exist.
C-propeptide of type II procollagen (sCPII)	Collagen synthesis	Yes	High levels of sCPII have been previously associated with a reduced risk (OR 0.53, 95% Cl 0.30-0.94) of ROA compared with no OA (Cibere et al. 2009); sCPII decreased (-7.4%) with the onset of OA (K/L 2 versus K/L 1) (Ishijima et al. 2011). High levels may be protective. Small decreases in sCPII may be effective at distinguishing early OA changes.
C-telopeptide of type II collagen / Collagen synthesis (uCTX-II/sCPII)	Collagen degradation to synthesis ratio	Yes	uCTX-II/sCPII increased (+134.3%) with onset of OA (K/L 1 versus K/L 2) (Ishijima et al, 2011); it was concluded that this biomarker may be particularly effective at identifying early OA compared with more established markers.
Collagen type II specific neoepitope (CIIM)	Collagen specifically degraded by MMP	Yes	Mean serum CIIM was significantly higher (+12.8%) in OA patients with mild OA and severe OA (+19.7%) compared to healthy controls (Bay-Jensen et al. 2011).
N-propeptide of type IIA procollagen (PIIANP)	Synthesis of type IIA collagen	Yes	73.0% lower in OA, and 42.5% lower in RA patients compared to controls (Rousseau et al. 2004); Very low serum PIIANP has been associated with an increased loss of medial cartilage volume and increased risk of joint replacement (Berry et al. 2010); However, patients with serum PIIANP in the highest quartile of the 5-year average had a significantly higher risk of progression than the other patients [relative risk (95% CI): 3.2 (1.1–9.2)] (Sharif et al. 2007).

BMI = body mass index; JSN = joint space narrowing; K/L = Kellgren and Lawrence system; MRI = magnetic resonance imaging; OA = osteoarthritis; PROA = pre-radiographic osteoarthritis; RA = rheumatoid arthritis; ROA = radiographic osteoarthritis; SF = synovial fluid; TKR = total knee replacement; OR = Odds ratio; CI = Confidence interval

Continued

Biomarker	Marker of.	Commercially avail. ELISA for urine or serum	Summary
		Biomarkers related to aggrecan metabolism	
Aggrecan	A major proteoglycan in articular cartilage.	No	Serum concentrations were 62.9% higher in serum of OA patients compared to controls as well as positively correlated with MRI score (r = 0.422), age (r = 0.350), BMI (r = 0.399) and disease duration (r = 0.499) (El-Arman et al. 2010). Resting concentrations are unchanged following 30 min of exercise (Helmark et al. 2012). However, limited acute exercise studies with immediate blood sample collection exist.
Aggrecan ARGS	A fragment from the aggrecanase cleavage at the ^{392}Glu-^{393}Ala bond in the interglobular domain of aggrecan	No	Levels of SF aggrecan ARGS fragments are increased following acute knee injury and acute inflammatory arthritis (Larsson et al. 2009). Aggrecan ARGS is also understood to be more sensitive measure than total aggrecan; Serum ARGS neoepitope concentrations were elevated in OA-TKR subjects compared to non-surgical OA and healthy subjects, however, serum aggrecan ARGS did not distinguish between non-surgical OA and healthy and was not associated with number of OA joints or WOMAC score (Germaschewski et al. 2014).
Serum Keratan Sulphate (sKS)	Marker of cartilage degradation	Yes	Elevated in OA patients (+49.0%) and following recent knee injury (+78.9%) (Wakitani et al. 2007). Resting serum have been shown to be elevated (+21.0%) in athletes (Roos et al. 1995). Increases in sKS following acute running exercise (+5.6%) (Roos et al. 1995). Limited acute exercise studies exist.
Aggrecan chrondroitin sulfate epitope CS846	This marker may reflect newly synthesised aggrecan	No	Elevated in joint fluid of individuals with knee injuries and OA (Lohmander et al. 1999). However, differences in serum concentrations between healthy and OA patients was not observed in serum, even when adjusted for age, sex and BMI (Cibere et al. 2009).

BMI = body mass index; JSN = joint space narrowing; K/L = Kellgren and Lawrence system; MRI = magnetic resonance imaging; OA = osteoarthritis; PROA = pre-radiographic osteoarthritis; RA = rheumatoid arthritis; ROA = radiographic osteoarthritis; SF = synovial fluid; TKR = total knee replacement

Continued

Biomarker	Marker of.	Commercially avail. ELISA for urine or serum	Summary
		Biomarkers related to non-collagenous proteins	
Cartilage oligomeric matrix protein (COMP)	Cartilage degradation / turnover	Yes	2.8-50.8% greater in knee OA (Mundermann et al. 2009; Wakitani et al. 2007), 12.5-123.6% higher in RA (Neidhart et al. 1997; Law et al. 2015), and 41.7-122.7% greater following knee injury (Kuhne et al. 2014; Wakitani et al. 2007) compared to healthy controls; serum COMP has been correlated with SF concentrations (r=0.46 - 0.58) (Kuhne et al. 2014; Lohmander et al. 1994, respectively); radiographic severity (r=0.370) and MRI score (r=0.654) of knee OA (El-Arman et al. 2010), as well as changes in JSN over 3 years (r = 0.372) (Vilim et al. 2002). Serum COMP increase between 5.6% (walking) and 194% (ultra-running) following exercise (Denning et al. 2016; Shin et al. 2012); correlated with the total decrease in knee cartilage volume (r = 0.487) (Kersting et al. 2005), and may be of prognostic value (Erhart Hledik et al. 2012).
Hyaluronan (HA)	Cartilage degradation and synovial inflammation	Yes	26.0-107.7% greater in knee OA (Elliot et al. 2005; Garnero et al. 2001) and 130.7% greater in RA patients (Engström-Laurent and Hällgren, 1987) compared to healthy controls; Greater amonmg individuals with highest level of OA progression (Sharif et al. 1995a); positively correlated with the severity of OA (r=0.410) (Inoue et al. 2011) and the rate of JSN over last 5 years (r=0.404) (Sasaki et al. 2013). Increases of between 13-158% have been shown following acute exercise (Roos et al. 1995; Engström-Laurent and Hällgren, 1987), particularly in RA patient population (Engström-Laurent and Hällgren, 1987).

BMI = body mass index; JSN = joint space narrowing; K/L = Kellgren and Lawrence system; MRI = magnetic resonance imaging; OA = osteoarthritis; PROA = pre-radiographic osteoarthritis; RA = rheumatoid arthritis; ROA = radiographic osteoarthritis; SF = synovial fluid; TKR = total knee replacement

From the previously identified biomarkers, serum cartilage oligomeric matrix protein (COMP) and serum hyaluronan (HA) were selected as biomarkers for this thesis. COMP an HA were selected based on several considerations, including, the understanding that structural molecules or fragments are the most likely to be the best candidates as biomarkers (Lotz et al. 2013). Moreover, both COMP and HA have been successfully investigated in both synovial fluid and in serum. Higher concentrations of both COMP and HA are indicative of synovial fluid, suggesting that these markers originate from the joint (Catterall et al. 2010; Pitsillides et al. 1994; Saxne et al. 1992). Synovial fluid is, however, difficult to obtain in healthy individuals and therefore the ability to assess these markers in serum samples was imperative. Importantly, serum COMP and HA are attractive biomarkers given the strength of performance in line with "BIPED" categories (Van Spil et al. 2010), the fact they have both been successfully investigated in healthy individuals and following acute loading, and given that an enzyme-linked immunosorbent assay (ELISA) are commercially available and have been previously referenced in academic journals.

In addition to serum COMP and HA, a novel biomarker that has demonstrated potential is lubricin. Lubricin is a chondroprotective lubricating glycoprotein and product of the proteoglycan 4 (PRG-4) gene that was originally identified by Swann et al. (1981) (HGNC:9364) and is often referred interchangeably to the homologous molecules known as superficial zone protein (SZP) and proteoglygan 4 (PRG-4) (Jones and Flannery, 2007). This novel biomarker was not identified in the initial review as it was focussed on human studies, and, to date, studies on lubricin have predominantly been conducted in animal samples. However, a previous review of biochemical markers recognised lubricin, together with HA, as having an important role in the lubrication of the joint (Garnero et al. 2000). Lubricin is understood also to be present as a thin layer on the surface of normal articular cartilage; however, the relative contribution of surface lubricin from chondrocytes and synoviocytes remains unknown (Schumacher et al. 2005). In addition, lubricin has also been localised to other knee joint structures including the meniscus (Schumacher et al. 2005), tendon (Rees et al. 2002) and ligament (Sun et al. 2006). The distinct functional domains in lubricin have been suggested to regulate multiple biological functions, including boundary lubrication and the reduction of friction associated with joint movement (Jay et al. 2007b). As a biomarker, a reduction in lubricin may indicate reduced joint lubrication and an increased risk of degenerative change. In animal models, synovial fluid that lacks lubricin has been shown to demonstrate early and higher levels of friction, coupled with wear at the cartilage surface (Jay

et al. 2007b). Moreover, animal studies have also indicated that lubricin synthesis is down-regulated in degenerative joints (Abusara et al. 2013; Ni et al. 2011) as well as following ACL injury. In addition, the mutation / interference of lubricin gene expression provokes CACP (camptodactyly-arthropathy-coxa-vara-pericarditis) syndrome in humans, which is an autosomal recessive disease resulting in non-inflammatory synovial hyperplasia and fibrosis and results in premature joint failure (Marcelino et al. 1999). Together, these indicate that reduced lubricin expression may predispose cartilage to damage. A recent proteomic analysis of knee synovial fluid from normal and OA patients has also demonstrated a statistically significant association between lubricin and joint space narrowing (Ritter et al. 2014). Given that lubricin had demonstrated early promise as a potential biomarker in both animal studies and more recently in human synovial fluid, it was decided that this PhD should also explore serum lubricin as a biochemical marker.

2.10 Serum biomarkers; normative values in health and disease

Overall, serum COMP has been the most highly researched biomarker, offering the greatest amount of normative data. Studies reporting serum COMP concentrations in healthy individuals have typically reported values within a range of 109 – 1724 ng/ml (Denning et al. 2014; Hoch et al. 2012). However, higher mean baseline values of 3300 ng/ml and 7100 ng/ml have been reported in a group of healthy older individual and healthy runners, respectively (Neidhart et al. 2000). The higher serum COMP concentrations previously reported in marathon runners may result from differences such as the onset of early degenerative joint change and/or increased training history. However, differences may also relate to methodological differences such as the inclusion criteria and exercise restrictions before the blood sampling. Several clinical populations have demonstrated elevated COMP compared to healthy controls, including OA (Neidhart et al. 1997) and RA (Law et al. 2015). Serum concentrations have been shown to range between 890 – 5700 ng/ml in OA patients (Jordan et al. 2003; Neidhart et al. 1997), and between 1349 – 7200 ng/ml in RA patients (Law et al. 2015; Neidhart et al. 1997). Increases are understood to reflect increased level of cartilage degradation indicative of joint disease and provide both a diagnostic and prognostic marker of knee OA progression (Verma and Dalal, 2013; Vilim et al. 2002). Serum COMP concentrations have been found to remain constant during the daytime in patients with RA

and OA (Andersson et al. 2006a; Kong et al. 2006). However, serum COMP has been found to decrease significantly during the night in OA and RA patients, suggesting the rapid removal of COMP from the circulation during overnight joint unloading (Andersson et al. 2006a).

Fewer studies have explored serum HA concentrations in humans compared to serum COMP. Studies reporting serum HA concentrations in healthy individuals have reported mean values within a range of 26 – 75 ng/ml (Engström-Laurent and Hällgren, 1987; Pruksakorn et al. 2013; Wakitani et al. 2007). The reference range of HA in serum has previously been reported as between 10-100 ng/ml (Engström-Laurent et al. 1985). However, higher mean baseline values of between 69 - 258 ng/ml and between 124 - 402 ng/ml have been reported in a OA (Criscione et al. 2005; Wakitani et al. 2007) and RA patients (Engström-Laurent and Hällgren, 1987), respectively. In OA patients, decreases in serum concentrations during the night are followed with a significant early morning increase, with the latter appearing to be related to morning activities (Criscione et al. 2005). However, like serum COMP, serum HA appears to remain stable during the day in healthy individuals, as well as in OA and RA patients (Lindqvist et al. 2010; Manicourt et al. 1999). The paucity of normative values for serum HA in healthy individuals, as well as in clinical population emphasises the need for the present work. Considerable variability exists in serum COMP and serum HA biomarkers in both healthy and clinical populations. Serum HA has previously been shown to be influenced by various individual factors, including sex, with higher serum HA concentrations typically found in men compared to women (Elliott et al. 2005). However, overall, little is known regarding how lifestyle factors such as age, weight and physical activity affect serum concentrations. Elucidating theses factors that are important for the use of these biomarkers, as well as for the greater understanding of joint health.

In contrast to serum COMP and HA, human serum lubricin concentrations have not been reported within the literature. Studies that have investigated lubricin have been either *in vitro* animal or *in vivo* animal studies. However, these studies have indicated that lubricin is a promising biomarker, which may in future be used as a marker of joint lubrication and an indicator of joint OA. Developing the pool of normative data values is imperative for an improved understanding of lubricin and for its use as a biomarker.

2.11 The effect of ageing on serum biomarkers

Several studies have demonstrated that ageing is associated with increases in serum COMP concentrations (Clark et al. 1999; El-Arman et al. 2010; Jordan et al. 2003; Verma and Dalal, 2013). For example, in a sample of individuals (age ≥ 45 years) with OA (n = 143) and matched controls (n = 148), serum COMP was found to be significantly elevated in individuals aged 65 or above (1302 ± 497 ng/ml) versus individuals aged between 45-54 and 55-64 years (1058 ± 432 and 1039 ± 313 ng/ml, respectively) (Clark et al. 1999). Interestingly, when age differences were examined by sex, Clark and colleagues (1999) found that the effect of age was more prominent in females compared to men. In a similar study, serum COMP was also found to have a positive linear association with age in both OA (n = 100; age 40-80 years) and control participants (n = 50; age: 40-60 years) (Verma and Dalal, 2013). However, serum COMP was reported to remain relatively constant until 50 years of age. In the OA group, there was a large increase in serum COMP in individuals above 60 years of age compared to younger OA sufferers (40-60 years).

Serum HA concentrations have been correlated with age (r = 0.676) in a large sample (n = 616; age range: 20-85) of both OA patients and controls (Inoue et al. 2011). Data from the healthy individuals demonstrated that serum HA increased from 35 ± 8 ng/ml in individuals in their twenties, to 127 ± 54 ng/ml in their eighties. While this increase was comparable in participants with knee OA, serum HA levels of the knee OA group were significantly higher in adults aged in their forties and seventies compared to the younger adult group. This may indicate a greater initial age-related change in serum HA among individuals with knee OA, which then levels out, before becoming more prominent again in later life. In a similar study comprising of 753 participants, including 298 without radiographic knee or hip OA, and 455 with radiographic knee OA, mean serum HA levels were also found to be correlated with age (r = 0.35) (Elliott et al. 2005).

As previously discussed, there is currently no data relating to serum lubricin in humans. However, a recent study demonstrated that older rats exhibited reduced lubricin expression compared to younger rats (Musumeci et al. 2014). Furthermore, this study demonstrated that moderate physical exercise and mechanical stimulation, improved lubrication, prevented

cartilage degeneration, promoted lubricin synthesis in synovial fluid and ameliorated the age-related reduction compared to unexercised rats.

2.12 The effect exercise on serum biomarkers

Knee joint loading through acute exercise results in acute increases in serum COMP (see Table 2-2). The response of serum COMP to exercise is understood to relate to an increase in cartilage metabolism (Neidhart et al. 2000) and has been correlated independently with both with decreases in cartilage volume (Kersting et al. 2005) and changes in cartilage thickness over a 5-year period (Erhart Hledik et al. 2012). It has been suggested that the serum COMP response to loading may have the potential to be used to determine the health of cartilage and to detect underlying pathology of OA. Acute exercise results in a 'dose dependent' increase in COMP (Kersting et al. 2005; Kim et al. 2009; Mündermann et al. 2005; Neidhart et al. 2000; Niehoff et al. 2010), which usually returns to baseline level within 30 minutes of cessation of activity (Andersson et al. 2006b; Mündermann et al. 2005), but may remain elevated for up to 2 days following a marathon and up to 6 days following an ultra-marathon (Kim et al. 2009). Acute increases in serum COMP have largely be observed following walking (Mündermann et al. 2005) or running exercise (Kim et al. 2009; Neidhart et al. 2000), however, high intensity circuit training (Andersson et al. 2006) and drop jumps (Niehoff et al. 2011) have been shown to elevate serum COMP in OA patients and healthy participants, respectively. Twelve weeks of running training has also been shown to reduce the exercise response of serum COMP to acute walking, indicating that training status may influence the serum biomarker response to acute exercise.

In addition, serum HA has also been shown to increase modestly following a short maximal cycle test, but not following 20 minutes of moderate intensity physical exercise led by a physiotherapy (Engström-Laurent and Hällgren, 1987). In contrast, moderate acute exercise has been shown to result in large serum HA increases in RA patients (Engström-Laurent and Hällgren, 1987). This suggest that the magnitude of response may be related to the status and health of joints. In a separate study, plasma HA has been shown to rise with exercise time and demonstrate an exponential increase with increasing exercise intensity in healthy individuals (Hinghofer-Szalkay et al. 2002). Compared to serum COMP, fewer studies relating to the effect of acute exercise on serum HA currently exist (see Table 2-3).

The effect on biomarkers following exercise training (see Table 2-4) has been investigated in healthy individuals (Celik et al. 2013; Liphardt et al. 2009), as well as in patients with OA (Andersson et al. 2006; Hunt et al. 2013) and RA patients (de Jong et al. 2008; Law et al. 2015). Twelve weeks of running, cycling and swimming training in healthy individuals resulted in a non-significant decrease in baseline COMP of 3.1%, 3.1%, and 3.4%, respectively (Celik et al. 2013). Moreover, serum COMP remained unchained following a 6-week exercise intervention in OA patients (Andersson et al. 2006b), and following an 8-week exercise intervention in RA patients (Law et al. 2015). Furthermore, in an exercise and weight loss intervention in OA patients, both serum COMP as well as serum HA remained relatively stable during the 18-month intervention period (Chua et al. 2008). Despite differences in the exercise protocol between studies, results suggest that the effect of exercise training on baseline COMP may be small, typically resulting in a decrease in serum COMP of between 3.1% - 7.2%. Interestingly, although exercise training does not appear to have a large impact on serum COMP, the effect of not exercising for patients with OA has been associated with an increase in serum COMP, which may signify an increase in cartilage degradation. Moreover, in healthy young footballers, serum COMP has been shown to increase (albeit small) across the course of an athletic season (Hoch et al. 2012), indicating that high levels of continuous exercise training may lead to an increase in baseline serum COMP. This supports previous research that have reported that runners have higher baseline COMP compared to control subjects (Neidhart et al. 2000). Therefore, it is possible that while moderate levels of exercise are important for cartilage health, inactivity and sustained chronic exercise training may have an adverse effect on joint health.

Moreover, to date, there are no studies that have explored the effect of acute exercise or chronic exercise training on serum lubricin. However, during the embryonic development of the mouse elbow joint, PRG-4 messenger ribonucleic acid (mRNA) expression begins at the onset of joint cavitation (Rhee et al. 2005), indicating that the expression may be induced by the initiation of relative motion between the articular surfaces. Similarly, using cartilage explant cultures, Nugent et al. (2006) demonstrated that both static and dynamic shear stress increased cartilage secretion of lubricin compared to controls. Continuous passive motion also regulated lubricin biosynthesis, increasing chondrocyte expression and synthesis when assessed *in vitro* in a bovine joint (Nugent-Derfus et al. 2007). Interestingly, this regulation was specific to regions of the femoral condyle that were sliding against the meniscus and tibial cartilage. Moreover, in a study to assess the dose response of muscular loading, lubricin

concentration from the intact knee joint of live mice was found to be significantly increased following acute increases in knee joint loading; however, these concentrations were observed to decrease with repeated ‘‘high’’ intensity loading (Abusara et al. 2013). Therefore, acute joint loading appears to be associated with increased expression of lubricin. This suggests a positive role of exercise on joint lubrication and cartilage health. Whether these promising findings can be found in humans is an important question that requires investigation.

In relation to exercise training, an 8-week exercise training study compared three different exercise intensities on gene expression of lubricin in young rats (Ni et al. 2012). All rats performed 60 minutes of running 5 days / week, with the low intensity group running at 15.2 m/min at a 0° gradient, the moderate group at 19.3 m/minute at a 5° gradient, and the high intensity group at 26.8 m/minute at a 10°gradient. This study demonstrated that both a low and moderate intensity running resulted in a significant increase in gene expression of lubricin in the cartilage of young rats (Ni et al. 2012). In contrast, both the high intensity and control groups demonstrated significantly greater osteoarthritic cartilage as assessed by the MANKIN score (a widely used histological evaluation of OA), which was also accompanied by significantly lower expressions of the lubricin gene. This study supports research demonstrating that strenuous joint exercise results in cartilage degeneration similar to early OA changes in normal rats (Pap et al. 1998). In ACL injured animal models, moderate exercise has been found to provide a protective effect on articular cartilage, however, this disappeared with intense exercise (Galois et al. 2004). Similarly, forced exercise following injury significantly reduced lubricin expression and increased osteoarthritic changes compared to injury alone (Elsaid et al. 2012). This increased cartilage degradation was associated with increased levels of capesin-3, which is a known potent activator of chrondrocyte apoptosis during OA development. Together, this research suggests some evidence of a ‘U-shaped curve’, with inactivity and high intensity or strenuous exercise, particularly in an already comprised joint (i.e. ACL injured knee) linked to adverse changes to lubricin expression.

The acute the response of joint biomarkers to loading may have the potential to be used to determine the health of cartilage and to detect underlying pathology of OA, however, to date, this is largely under explored. This is particularly true for biomarkers other than serum COMP, such as serum HA and lubricin. More research is required to improve the understanding of the effect of exercise modality on the acute exercise response, as well as the

effect of age, gender, training status, the response of injured vs non-injured, and healthy vs individuals with symptomatic knee OA. Moreover, little is known regarding the effect of chronic exercise training on serum biomarkers, particularly relating to serum HA and COMP. This emphasises the importance of the present research in developing the understanding of the role of exercise on these serum joint markers. Many questions remain regarding whether the volume, intensity and type of exercise training influence these markers of joint health.

Table 2-2 A summary of studies investigating the acute effects of exercise on serum COMP

Author, year	Participants	Age (range/ mean ±SD)	Exercise intervention	Blood sampling	Change in biomarker	Summary
Neidhart et al. (2000)	8 healthy marathon runners	25-34	Marathon run	31km, 42km, 2h post, 24h post, 48h post	15.5% ↑ at 31km*, 23.9% ↑ at 42km*, 28.2% ↑ at 2h post*, returned to baseline 24-48h post run	Significant ↑ in sCOMP following running, which remained elevated for > 2 h.
Kersting et al. (2005)	18 healthy adults	32 ± 8	1h training run (at max sustainable speed)	25-min post, 2.5h post	4.4% ↑ at 25-in post (NS), returned to baseline 2.5 h post	1h run did not significantly increase sCOMP. Change in cartilage volume and sCOMP demonstrated significant correlation.
Mundermann et al. (2005)	10 healthy adults	26-38	30-min walk	Immed. post, 0.5h, 1.5h, 3.5h and 5.5h post	9.7% ↑ Immed. post**, returned to baseline at 0.5 h, 8.2% ↑ at 5.5 h post**	Mod intensity walking resulted in significant ↑ sCOMP, which returned to baseline at 0.5h post. A second significant ↑ at 5.5hr post suggest a potential metabolic delay.
Andersson et al. (2006)	7 adults with knee OA	36-65	1h of high intensity circuit training exercise	Immed. post, 0.5h, 1h, 2h, 3h, 4h and 5h post	10.5% ↑ immed. post* Returned to baseline at 0.5h post and continued to decrease	1 h of high intensity training significantly ↑ sCOMP and subsequently ↓ following rest.
Kim et al. (2009)	10 male marathon runners	44-5	Marathon run	1-2h pre-race, 0km, 10km, 20km, 30km, 42.2km, daily for 6d	1.6-fold (60%↑) at 10km** with little change thereafter. At 2d post sCOMP had returned to baseline	Running greater distances may induce more impact stress on cartilage tissue. Recovery time may vary with running distance.
	10 male ultra-marathon runners	45-59	Ultra-marathon run (200 km)	6-10h pre, 100km, 200km, daily for 6d	1.9-fold (90%↑) at 200km**, remained elevated for 3d, returned to baseline following 6d	

d = day; h = hour; HI = high-intensity, HRmax = maximum heart rate; Immed. = Immediately; km = kilometre; max = maximum; min = minute; NS = non-significant; reps = repetitions; RT = resistance training; sCOMP = serum COMP; SF = synovial fluid; ↓ = decrease; ↑ = increase; * = $P < 0.05$; ** = $P < 0.01$

Continued

Author, year	Participants	Age (range/ mean ±SD)	Exercise intervention	Blood sampling	Change in biomarker	Summary
Münderman n et al. (2009)	42 patients with knee OA, 41 healthy age-matched controls	40-72	30-min walk at self-selected speed	Immed. post, 0.5h post, 1.5h post, 3.5h post, and 5.5h post	6.3% ↑ and 5.6% ↑ in sCOMP immed. post-exercise in OA** and healthy**, respectively. sCOMP returned to baseline at 0.5h post and continued ↓ with rest. Change in COMP NS between groups	sCOMP increases significantly following walking in OA patients and healthy controls. No change between group. sCOMP change not related to ambulatory load.
Niehoff et al. (2010)	5 healthy males	26-28	Randomised, cross-over design: a) High-impact running b) Slow, deep knee bends c) Lymphatic draining d) Rest (all 30 mins)	Pre-exercise, immediately post, 5, 10, 15, 20, 30, 50, 70, 90, 120, 150, 210, 240, 270, 300, 330, 360, 390, 420 min post.	a) 39% ↑ ** immediately-post, remained ↑ up to 90 min-post, then returned to pre-exercise b) No change c) No change d) 19% ↓ **	The elevation of sCOMP appears to depend on the loading mode of the physical activity.
Helmark et al. (2010)	29 females with knee OA	Group a) 67 ± 7 Group b) 66 ± 6	Two group design: a) No-exercise group b) RT group: Leg extensions (10 rep x 25 sets) starting every 1.5 min (~ 50 min)	0.5h after arrival, after 4h microdialysis, 0.5h post-exercise, after 4h microdialysis.	↓ in sCOMP * in both groups over time. ↓ in intra-articular COMP over time* in exercise group but not in no-exercise group.	Exercise did not ↑ sCOMP. However, sCOMP did ↓ with rest.

d = day; h = hour; HI = high-intensity, HRmax = maximum heart rate; Immed. = Immediately; km = kilometre; max = maximum; min = minute; NS = non-significant; reps = repetitions; RT = resistance training; sCOMP = serum COMP; SF = synovial fluid; ↓ = decrease; ↑ = increase; * = $P < 0.05$; ** = $P < 0.01$

Continued

Author, year	Participants	Age (range/ mean ±SD)	Exercise intervention	Blood sampling	Change in biomarker	Summary
Niehoff et al. (2011)	7 male, 7 female healthy sedentary participants	18-26	Randomised, cross-over design: a) 100 vertical drop- landings (30 min) b) Running at 4.9mph (30 mins) c) 30 min resting in chair	Immed. post, 0.5h, 1h, 2h and 3h post	a) 32.3% ↑** immed. post b) 30.7% ↑** immed. post Returned to baseline at 2h post-exercise following both exercise modalities. c) No change	No difference between the elevation magnitude or duration of sCOMP between modalities of exercise. Cartilage deformation more pronounced after running compared to drop-jumps.
Helmark et al. (2012)	11 patients with knee OA	61±11	30 min one-legged knee- extension exercise	Pre-exercise, 15-30 min post-exercise	No significant change	No significant changes in sCOMP following exercise. Concentration of COMP in SF reduced significantly.
Erhart-Hledik et al. (2012)	17 patients with knee OA	59 ± 9	30-min walk at self-selected speed	Immed. post, 3.5h, and 5.5h post	4% ↑ Immed. post exercise (NS). Followed by decrease; ↓ 16.3%* at 5.5h post.	Small increase followed by significant post exercise decrease in serum COMP. Higher relative post-activity sCOMP levels were associated with ↑ reduction in cartilage thickness over 5-year follow-up.
Shin et al. (2012)	20 Healthy runners	51.5 ± 6.5	308 km ultra-marathon	100 km, 200 km, and 308 km	130.7% ↑* at 100 km, 160.4% ↑*** at 200 km, and 194.1% ↑*** at 308 km	Ultra-marathon running has a major impact on cartilage turnover. Greater distances result in more tissue turnover / damage.

d = day; h = hour; HI = high-intensity, HRmax = maximum heart rate; Immed. = Immediately; km = kilometre; max = maximum; min = minute; NS = non-significant; reps = repetitions; RT = resistance training; sCOMP = serum COMP; SF = synovial fluid; ↓ = decrease; ↑ = increase; * = $P < 0.05$; ** = $P < 0.01$

Continued

Author, year	Participants	Age (range/ mean ±SD)	Exercise intervention	Blood sampling	Change in biomarker	Summary
Celik et al. (2013)	44 Healthy men: 12 run trained; 11 cycle trained; 12 swim trained; and 12 controls	18-25	30 min walk (5 km/h on a treadmill with a 1.5% incline).	Pre, Immed. post, and 0.5h post	Post exercise there was a 15.8% ↑ (NS) in run trained 26.1%↑* in cycle trained 30.5%↑* in swim trained 28.4% ↑*in control group	12 weeks of regular, weight-bearing, high-impact running decreases the acute sCOMP response to 30min of walking.
Pruksakorn et al. (2013)	86 healthy adults (58 exercise group; 24 controls)	19-21	14 km walk: a) experimental group walked at incline of 5.97°, and controls on level)	Pre (fasted), within 1h post exercise, 20 h after 1st sample.	25.3% ↑* following incline walking, 7.1% ↑ (NS) following flat walking. Recovery at 20 hr post	sCOMP increases are greater with an increased exercise load (via elevation in walking gradient).

d = day; h = hour; HI = high-intensity, HRmax = maximum heart rate; Immed. = Immediately; km = kilometre; max = maximum; min = minute; NS = non-significant; reps = repetitions; RT = resistance training; sCOMP = serum COMP; SF = synovial fluid; ↓ = decrease; ↑ = increase; * = $P < 0.05$; ** = $P < 0.01$

Table 2-3 A summary of studies investigating the acute effects of exercise on serum and plasma HA

Author, year	Participants	Age (range/ mean ±SD)	Exercise intervention	Blood sampling	Change in biomarker	Summary
Engstrom & Hallgren (1987)	9 healthy men 7 with RA	26-43 48-76	4 men cycled 300 W time to exhaustion test; 5 men performed incremental cycle test; 7 RA patients completed 20 min of physiotherapy	Pre-exercise and immed. post	147%↑* in sHA following exercise in healthy men and 158% ↑ in patients with RA.	Significant ↑ in sHA in healthy subjects who performed heavy exercise and in RA patients who performed moderate exercise. Absolute increase was much greater in RA patients.
Roos et al. (1995)	33 healthy athletes: recreational runners; soccer players; LD runners	Recreational runners (32 years); soccer players (24 years); LD runners (31 years)	Recreational runners ran on a treadmill for 60 min; soccer players played 90 min of soccer; LD runners ran 21kin (75-85min) on road.	Pre-exercise and 0.5-1h post exercise	13% ↑ (NS) post exercise (athletes pre-post values were grouped). No differences between exercise modalities.	In athletes, exercise of between 60-85 min does not increase sHA.
Hinghofer -Szalkay et al. (2002)	6 healthy men	29±1	Cross-over design: 3 interventions a) MAX 15min cycle); b) SUBMAX 30 min cycle; c) MOD 30 min cycle	Every 5 min during MAX, every 10 min during SUB and every 30 min during MOD; plus 15 and 45 min after MAX, 30 and 60 min after SUB and MOD, respectively	76 %↑* in pHA following MAX exercise, by 44%↑* SUBMAX exercise, and 27% ↑ following MOD exercise. pHA ↓ by 43%* and by 36%* below resting levels after MAX and SUBMAX, respectively.	pHA increased significantly following MAX and SUBMAX exercise. Post-exercise pHA decrease was proportional to the exercise-induced pHA increase, suggesting elevated HA clearance with rising plasma levels after physical exertion.
Pruksakorn et al. (2013)	86 healthy adults (58 exercise group; 24 controls)	19-21	14 km walk: a) experimental group walked at incline of 5.97°, and controls on level)	Pre (fasted), within 1h post exercise, 20 h after 1st sample.	↓ 50.2% (NS) in experimental group and 19.9% (NS) in controls. 20h post exercise, sHA ↑* significantly from 1h post concentration.	Although NS, sHA decreased after exercise, prior to returning to baseline 20 hr post activity.

d = day; h = hour; Immed. = Immediately; LD = long distance; MAX = maximal; min = minute; MOD = moderate; NS = non-significant; pHA = plasma hyaluonan; RA = rheumatoid arthritis; reps = repetitions; sHA = serum hyaluronan; SUBMAX = sub maximal; W = Watts; ↓ = decrease; ↑ = increase; * = $P < 0.05$; ** = $P < 0.01$; = degrees

Table 2-4 A summary of studies investigating the chronic effects of exercise training on serum COMP

Author, year	Participants	Age (range/ mean ±SD)	Study design	Blood sampling	Change in biomarker	Summary
Andersson et al. (2006)	58 OA patients	36–65	RCT with 6-week intervention: patients randomised to exercise or control. Exercise = 1h supervised x2 sessions/week (5 HI stations of weight-bearing exercises at > 60% HRmax) and 30min home- based exercises x5 sessions/week. Control = usual activities (no restrictions)	Pre and post 60 min of exercise/rest at 4 time points: (-3 weeks, 0 weeks, 6 weeks (during intervention) and at 24 weeks).	3 weeks: ↓ COMP in both groups after 1h rest*** Weeks 0 and 6: ↑ COMP post-exercise in EG *** ↓ COMP post 60mins rest in CG *** Week 24: ↓ COMP in both groups after 1h rest***	sCOMP ↑ with exercise and ↓ with rest. However, sCOMP did not differ between start (-3 weeks) and end of the study (24 weeks) in either group – there appears to be no effect of 6 weeks of training in OA patients.
De Jong et al. (2008)	281 RA patients	54 ± 16	RCT to either 2 years of HI exercise group or UC. HI exercise consisted of supervised 2 individualised sessions/week (20 bike at 70-90% HRmax; 20 min circuit training (8-10 exercises repeated 8-15 times); 20 min "sport or game" e.g. badminton, indoor soccer)	Baseline, 3 months, 2 years	Small sCOMP ↑ in HI exercise group, small sCOMP ↓ in control group (NS).	Supplementing usual care with HI exercise training did not significantly change sCOMP at 3 months or 2 years.
Chua et al. (2008)	193 overweight adults with knee OA	≥60	RCT with 18-month intervention: 4 groups – a) healthy lifestyle, b) diet, c) exercise, d) diet and exercise. Exercise group: x3 sessions / week: 15 min aerobic (50-75% HRR), 15 min lower-body RT (2 x 12 reps of 4 exercises), 15 min cool-down	Baseline, 6 and 18 months	No significant changes in sCOMP were found at 6 or 18 months.	Study showed that sCOMP levels were relatively stable during the 18-month intervention.

CG = control group; d = day ; EG = exercise group; h = hour; HDT = head down training; HI = high-intensity; HRmax = age-predicted maximum heart rate; HRR = heart rate reserve; MDC = minimal detectable change ; min = minute; NS = non-significant ; OA = osteoarthritis; reps = repetitions; RM = repetition maximum; RA = rheumatoid arthritis; RCT = randomised controlled trial; RT = resistance training; sCOMP = serum COMP, VT, vibration training; ↓ = decrease; ↑ = increase; * = $P < 0.05$; ** = $P < 0.01$; = degrees

Continued

Author, year	Participants	Age (range/ mean ±SD)	Study design	Blood sampling	Change in biomarker	Summary
Liphardt et al. (2009)	8 healthy males	26 ± 5	Randomised cross-over-design with 2 phases: training phase = 14 days of 6°-HDT with 2x5-min whole body vibration training/day; control phase = 14 days of 6°-HDT	3d and 1d pre-intervention, days 2, 6, 8, 11, 13, 14 of intervention. Days 2, 3, 5 of recovery.	14.8% ↓* COMP in control condition 10.1% ↓* COMP in training condition after 24h treatment. Difference in COMP change between treatments was NS. sCOMP returned to baseline after 1d recovery.	sCOMP is sensitive to unloading. VT did not prevent significant ↓ in COMP because of bed rest.
Petersen et al. (2010)	36 patients with knee OA	50-70	RCT with 12-week intervention: RT 3 sessions per week – warm-up, leg extension and leg press exercises (wk 0-7: 15-8RM 4sets x 12-8 reps; wk 7-12: progressing to 8RM 4-5 sets x 5-8 reps) and supplementation with a) glucosamine b) ibuprofen or c) placebo.	Pre and post 12-week intervention	13% ↓* COMP in glucosamine group. No change in ibuprofen or placebo groups.	Training when combined with glucosamine appeared to reduce sCOMP. However, 12 weeks of RT alone does not appear to change sCOMP.
Hoch et al. (2012)	29 healthy soccer players	19.6±1.2	5-month longitudinal cohort study. Details regarding training and matches were not specified.	Pre-season, mid-season, and post-season (February - May)	16.2% ↑** in sCOMP at mid-season compared to pre-season. 9.6% ↑** in sCOMP post-season compared to pre-season.	sCOMP levels increased in athletes over a course of a season. However, the differences in sCOMP levels did not reach the calculated MDC value.

CG = control group; d = day ; EG = exercise group; h = hour; HDT = head down training; HI = high-intensity; HRmax = age-predicted maximum heart rate; HRR = heart rate reserve; MDC = minimal detectable change ; min = minute; NS = non-significant ; OA = osteoarthritis; reps = repetitions; RM = repetition maximum; RA = rheumatoid arthritis; RCT = randomised controlled trial; RT = resistance training; sCOMP = serum COMP, VT, vibration training; ↓ = decrease; ↑ = increase; * = $P < 0.05$; ** = $P < 0.01$; = degrees

Continued

Author, year	Participants	Age (range/ mean ±SD)	Study design	Blood sampling	Change in biomarker	Summary
Celik et al. (2013)	48 healthy sedentary men	18-25	RCT with 12-week intervention: 4 groups a) swim b) run, c) cycle, and d) control groups. All exercise groups: x 3 sessions/wk: 40 min / day, 5-min warm-up, 30-min exercise at 60–70% HRR, 5-min cool-down.	Pre and post 12-week intervention	No change in sCOMP.	Exercise training or control conditions did not alter resting sCOMP concentrations.
Law et al. (2015)	9 RA patients	57 ± 14	8-week progressive exercise training intervention: Approx 1h supervised exercise x3 sessions/wk - 30 min aerobic interval exercise (3 min at 70-90%HRmax; 2 min at 40-50% HRmax); 30 min RT - 3 lower exercises (1 x 15 reps at half load, followed by 3 x 8 reps at 8RM)	1 h post exercise at week 1,4, and 8	No changes in sCOMP over 8-week period	Combined aerobic and resistance training in RA patients appears well tolerated with no adverse effects on cartilage.

CG = control group; d = day ; EG = exercise group; h = hour; HDT = head down training; HI = high-intensity; HRmax = age-predicted maximum heart rate; HRR = heart rate reserve; MDC = minimal detectable change ; min = minute; NS = non-significant ; OA = osteoarthritis; reps = repetitions; RM = repetition maximum; RA = rheumatoid arthritis; RCT = randomised controlled trial; RT = resistance training; sCOMP = serum COMP, VT, vibration training; ↓ = decrease; ↑ = increase; * = $P < 0.05$; ** = $P < 0.01$; = degrees

2.13 Thesis outline

Despite a push for individuals to remain physically active and to avoid a sedentary lifestyle, the impact of physical activity and exercise on articular health remains unclear. Engaging in high levels of physical activity has been historically associated with joint 'wear and tear', while certain activities high impact activities such as running, are often considered to be bad for the knee joint. However, in contrast, research also suggests that joint loading is important for positive cartilage health. Thus, several questions remain regarding the effect of physical activity on knee joint health. In recent years, numerous novel biomarkers have been identified for diagnosis, prognosis and monitoring of articular cartilage breakdown and joint lubrication. These serum markers are easily obtained, have been shown to be mechano-sensitive, and have the potential to be used to determine the response of cartilage to loading and to detect underlying pathology of OA. Thus, research involving these novel markers has the potential to develop current understanding of the role of physical activity and ageing on knee articular cartilage. Important questions remain regarding the amount and type of physical activity and its impact on articular cartilage, including whether physical activity can protect the joint from age-related change. This information would help address questions regarding whether too much exercise has a negative effect on articular health, or whether certain exercise may be preferential for positive cartilage health. Further knowledge within this area of research will help both clinicians and health and fitness professionals develop a greater understanding of how physical activity affects articular cartilage, as well as the risk of developing OA. Overall, the aim of this thesis is to enhance understanding and provide more evidence based exercise recommendation for healthy individuals across a range of ages; that can in future be investigated in individuals with existing joint diseases such as OA and RA.

2.13.1 Specific thesis aims:

- To assess the intra-session reliability and measurement precision using US in healthy human knee joints.
- To explore a heterogeneous sample of healthy individuals and extend the pool of normative data for both femoral cartilage thickness measurements assessed by ultrasonography and serum cartilage biomarkers. Secondary aims are to determine the effect of ageing, physical activity, and anthropometric variables on these markers.

- To explore the effects of an acute bout of running and cycling exercise on these cartilage markers; of interest is whether trained runners, whom are accustomed to weight-bearing exercise, would respond differently to trained cyclists.
- To explore and compare the effects of these cartilage markers an acute bout of high load low frequency exercise with a low load high frequency bout of aerobic exercise in healthy individuals; of additional interest are to determine whether females have reduced cartilage thickness, and display different serum biomarker profiles at baseline and in response to exercise.

3 CHAPTER 3. General methodology

3.1 Participants

Participants were excluded from study participation if they met any of the following exclusion criteria: (i) diagnosed OA, rheumatoid arthritis, or other inflammatory disease of the knee (ii) a previous knee injury within the last 5 years (including meniscus tear or ligament damage or tear) (iii) a history of knee malalignment (varus / valgus) greater than 15° (iv) recent fracture of lower extremity (within last 6 months). Participants taking medication or supplements understood to interact with knee cartilage were excluded, these included: current or prior use of lipid-lowering therapy (e.g. fibric acids, nicotinic acids, bile acid sequestrates, fish oils), angiotensin converting enzyme (ACE) inhibitors, angiotensin receptor blockers, corticosteroid injections, current or past use (single use within last week and/or daily prescription within last 3 months) of non-steroidal anti-inflammatory drugs (NSAID), current or past (within last four weeks) glucosamine and/or chondroitin supplementation use. Moreover, training within the 48 hours prior to testing was limited to 1 hour of moderate intensity training, while participants were required to refrain from completing any physical activity training within the 24 hours prior to testing. All experimental research was approved by the local Research Ethics Committee (School of Sport Health and Exercise Sciences (SSHES), Bangor University) and conducted in accordance with the Helsinki Declaration (2013). Written informed consent was obtained from all participants.

3.2 Primary outcome measures

3.2.1 Sonographic assessment and analysis of femoral cartilage thickness

The US assessment was performed using a 12 MHz linear-array probe (Esaote S.P.A. MyLab50 ultrasound, Firenze, Italy) and acoustic coupling gel (Aquasonic 100, Parker Laboratories, Inc, Fairfield, NJ, USA) following a period of between 15-30 minutes of seated rest. With participants lying in a supine position and with the knee maximally flexed, the superior margin of the patellar was located and a line was marked on the skin using a washable marker at the point immediately above the superior margin of the patellar and at 1 cm intervals in a superior direction. The transducer was placed in a supra-patella transverse

position, perpendicular to the bone surface and orientated to optimise the US image (Naredo et al. 2009; Özçakar et al. 2014). The location at which the cartilage thickness of the intercondyle notch appeared greatest was marked on the skin and recorded to enable the examiner to return the transducer to the exact location for all subsequent scans. The same researcher performed all ultrasonography scans following training by a consultant rheumatologist with expertise using this technique.

US images were analysed by 'Image J' software (Image J, National Institute of Health, Bethesda, MD, USA) to determine the minimal cartilage thickness. The distance from the thin hyperechoic line formed at the synovial space-cartilage border to the line formed at the cartilage-bone border was used to measure minimal cartilage thickness at the lateral condyle, medial condyle and intercondylar notch (Özçakar et al. 2014). Anatomic reference points used in the present study corresponded to the midpoint of the intercondyle notch and 1 cm apart in the medial and lateral directions were used as an estimate of the medial and lateral condyle cartilage thickness, respectively (see Figure 3-1). Naredo and colleagues previously demonstrated good reproducibility in femoral cartilage thickness measurement when using comparable anatomical reference points (Naredo et al. 2009). Prior to analysis, all images were de-identified by second researcher for blinded analysis. Based on the pixel resolution (15.8 pixels /mm) of the images captured by ultrasonography, the ImageJ software allowed images to be measured to an accuracy of greater than one-tenth off a mm, or more specifically, one pixel was equal to 0.06 mm. The cartilage thickness of each image was measured in triplicate and an average of the three measurements was used for all data analysis. As required, the image contrast was adjusted to assist in appropriately identifying the hyperechoic line formed at the synovial space-cartilage border to the line formed at the cartilage-bone border.

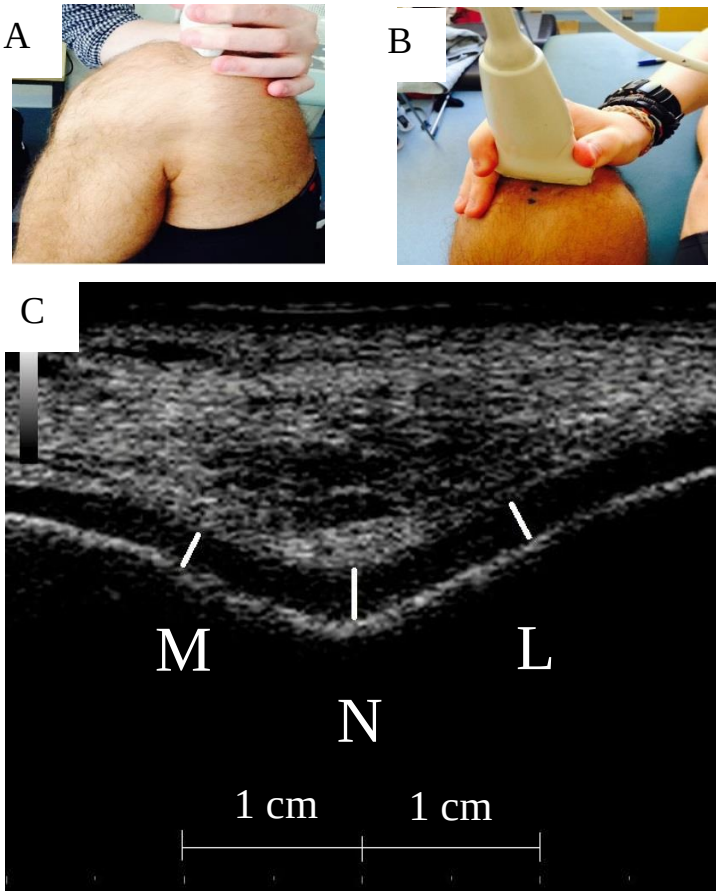


Figure 3-1 Images to show ultrasound assessment methodology. A) demonstrating supra patellar transverse placement of the US probe and B) anatomical marking to ensure correct placement and re-placement of US probe. C) US transverse image of the femoral articular cartilage; M = location of medial condyle; N = the intercondyle notch; L = the lateral condyle

3.2.2 Venous blood collection

Blood samples (6 ml) were obtained from an antecubital vein by a researcher trained in phlebotomy. Samples were collected using serum separator specific vacutainers (BD Vacutainer, Decton Dickinson & Co., Franklin Lakes, NJ, USA), allowed to clot for a period of 60 minutes at room temperature, prior to being centrifuged for 15 min at 1000 × gravity as specified by the ELISA kit inserts. Serum was subsequently aliquoted into eppendorf containers and immediately stored at -80°C until later analysis.

Serum COMP

Serum COMP was analysed using a commercially available sandwich ELISA (Human COMP ELISA kit KA0021, Abnova Corporation, Taiwan). Mean intra-assay coefficient of

variation (CV) was 5.39% and R^2 curve fit was > 0.99 across all analyses. Inter-assay CV was calculated as 2.20%. Briefly, 100 µl of standards and samples were added to the appropriate wells containing antibody specific for human COMP. The microplate was incubated on an orbital shaker (300 rpm) at 25°C for 1 hour. Wells were subsequently washed 3 times using appropriate wash solution prior to adding 100 µl of Biotin-antibody diluent to each well. Following a second incubation period (25°C for 1 hour at 300 rpm) and subsequent wash process (3-times), 100 µl of Streptavidin-Horse Radish Peroxidase was added to each well and incubated on an orbital shaker (300 rpm) for 30 minutes at 25°C. Following the final wash process, 100 µl of substrate solution was added to each well. The microtiter plate was covered with an adhesive strip and placed away from direct sunlight. Following a 15-minute incubation period the reaction was stopped by adding 100 µl of stop solution and the absorbance was determined using a microplate reader (Omega, BMG LABTECH, Ortenberg, Germany) set at 450 nm.

Serum hyaluronic acid

Serum hyaluronic acid was analysed using a commercially available competitive ELISA (Hyaluronic Acid (HA) ELISA Kit ABIN1873289, Cloud-Clone Corp, USA). Mean intra-assay CV was 6.82% and R^2 curve fit was > 0.99 across all analyses. Inter assay CV was calculated as 11.25%. Briefly, 50 µL each of standards and samples were added into the appropriate wells, prior to immediately adding 50 µL of Detection Reagent A. The microplate was incubated on an orbital shaker (300 rpm) at 37°C for 1 hour. Wells were subsequently washed 3 times with 350 µL of wash solution prior to adding 100 µL of Detection Reagent B working solution to each well. A second incubation period was provided (30 minutes at 37°C). Following incubation, the aspiration/wash process was repeated for a total 5 times as described previously. Ninety µL of substrate solution was then added to each well prior to a third incubate period (15 minutes at 37°C). Finally, 50 µL of stop solution was added to each well and the absorbance was determined using a microplate reader (Omega, BMG LABTECH, Ortenberg, Germany) set at 450 nm.

Serum lubricin

Lubricin was analysed using a commercially available sandwich ELISA (Human Proteoglycan 4 (PRG-4) ELISA kit CSB-E14124h, Cusabio Biotech Co, China). Mean intra-assay CV was 8.25% and R^2 curve fit was > 0.99 across all analyses. Inter assay CV was calculated as 5.37%. Briefly, 100 µl of standards and samples were added to the appropriate

wells containing antibody specific for PRG-4. The microplate was covered with an adhesive strip and incubated on an orbital shaker (300 rpm) at 37°C for 2 hours. 100 µl of Biotin-antibody diluent was added to each well and covered with a new adhesive strip. Following an incubation period (37°C for 1 hour at 300 rpm), the microplate was aspirated and washed and left to stand for 2 min for a total of 3 washes. 100 µl Horse Radish Peroxidase-avidin was added to each well, the microplate was covered and subsequently incubated on an orbital shaker (300 rpm) for 1 hour at 37°C. After repeating the aspiration/ wash process 5 times, 90 µl of Teramethylbenzidine (TMB) substrate solution was added to each well. The microtiter plate was covered with an adhesive strip and placed away from direct sunlight. Following a 25-minute incubation period the reaction was stopped by adding 50 µl of stop solution and the absorbance was determined using a microplate reader (Omega, BMG LABTECH, Ortenberg, Germany) set at 450 nm.

3.3 Secondary outcome measures

3.3.1 Anthropometrics

Body mass and body height was measured at using a calibrated balance beam scale (SECA, California, USA) and a wall-mounted tape measure (SECA, California, USA), respectively. Body mass index (BMI) was calculated by the equation: Body mass (in kilograms) / Height (in centimetres)^2.

3.3.2 Physical activity questionnaires

7 day long International Physical Activity Questionnaire (IPAQ)

To assess current levels of physical activity the International Physical Activity Questionnaire (IPAQ) 7-day (long version) questionnaire was used (Craig et al. 2003). The IPAQ 7-day questionnaire (see Appendix A) quantified the time spent being physically active in the last 7 days within the following domains: a) leisure time physical activity; b) domestic and gardening activities; c) work-related physical activity; d) transport-related physical activity. Responses within these domains were converted into a continuous measure [metabolic equivalent (MET) - minutes/week] as outlined by Craig et al. (2003). Participants were also grouped categorically according to their total level of physical activity into either ‘low’, ‘moderate’ or ‘high’ levels of physical activity by following public health guidelines for

physical activity (Pate, 1995). Individuals were grouped into the 'high' category if they met the following: a) completed vigorous-intensity activity on at least 3 days per week achieving minimum total physical activity of at least 1500 MET-min/week, or b) completed 7 or more days of any combination of walking, moderate-intensity or vigorous-intensity activities achieving a minimum total physical activity of at least 3000 MET-minutes/week. Individuals were grouped into the 'moderate' category if they met either of the following: a) completed 3 or more days of vigorous-intensity activity of at least 20 minutes per day, or b) completed 5 or more days of moderate-intensity activity and/or walking of at least 30 minutes per day, or c) completed 5 or more days of any combination of walking, moderate-intensity or vigorous intensity activities achieving a minimum total physical activity of at least 600 MET-minutes/week. Lastly, individuals who did not meet criteria for either 'moderate' or 'high' categories were considered to have a 'low' physical activity level.

Measurement of a Person's Habitual Physical Activity questionnaire

A modified version of the Measurement of a Person's Habitual Physical Activity questionnaire (Baecke et al. 1982), as used and validated by Pols et al. (1995), was also used to assess physical activity over the last 12 months (Appendix B). The modified version of the Measurement of a Person's Habitual Physical Activity questionnaire (Baecke et al. 1982) also evaluated physical activity across several domains, these included: a) work-related physical activity; b) sport related physical activity; c) leisure-related physical activity. Data collected within this questionnaire was then used to calculate a continuous measure in the form of a total physical activity index. Furthermore, in a similar approach to the IPAQ long questionnaire, participants were also grouped categorically according to their level of physical activity into either 'low', 'moderate' or 'high' levels of physical activity. However, in the absence of specific guidelines to categorise individuals into physical activity level groups, analysis of this questionnaire was conducted using data percentiles with the data divided into quartiles. Therefore, the 'high' group was represented by the values that are greater than or equal to the 75th percentile, the 'moderate' group by the inter-quartile range, and the 'low' group by the values that are less than or equal to the 25th percentile.

3.3.3 Maximum oxygen uptake (VO_2max)

Breath-by-breath analysis using an online gas analyser (Cortex, Leipzig, Germany) was used to determine VO_2max in both Chapter 5 and Chapter 6. The incremental exercise test was

either completed using a treadmill (HPcosmos Mercury 4 Med, Nussdorf-Traunstein, Germany) or cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). Specific protocol information can be found in the experimental protocol section of each chapter. Heart rate was monitored continuously using a heart rate monitor (Polar Electro, Kempele, Finland) and the rating of perceived exertion (RPE) was measured using the Borg scale (Borg, 1982) every 3 minutes. VO_2max was defined as the highest 30-s average in VO_2 and was accepted if two of the following were met; a plateau in VO_2 despite the continuation of exercise, a respiratory exchange ratio ≥ 1.05, or participants reached > 95% of predicted maximum heart rate. Alternatively, if the above criteria was not fulfilled or if participants completed a submaximal test, predicted VO_2 peak was calculated by extrapolating VO_2 to age predicted maximum heart rate (HRmax) using least squares regression (Astrand and Rodahl, 1977). For all studies VO_2max was expressed relative to body mass ($mL \cdot kg \cdot min^{-1}$). For sub-group analysis, each participants' VO_2max value was categorised into 'low', 'moderate' or 'high' groups. Utilising the classification proposed by Heywood (1998), individuals rated 'poor' or 'very poor' for their age and gender were grouped as 'low', individuals rated 'fair' or 'good' for their age and gender grouped as 'moderate', and individuals rated 'excellent' or 'superior' for their age and gender grouped as 'high'.

3.1 Statistical analyses

For all analyses data normality and normality of the model residuals were explored by the Shapiro-Wilk test and through visual inspection of Q-Q plots. Outliers were determined using z-scores and extreme outliers were removed as appropriate. If the assumption of normality was violated, data were transformed using the natural log function and reassessed for normality prior to proceeding with parametric analyses. All predictor variables were also assessed for multicollinearity using variance inflation factor (VIF) and tolerance statistics. All statistical models were also assessed for potential bias and appropriate conservative analyses were conducted as required. Figures and tables are presented as mean ± standard deviation (SD), with statistical significance set as $P < 0.05$ unless otherwise stated. Statistical analyses were performed using the SPSS 20.0 Software for Windows (SPSS Inc., Chicago, Ill., USA). Further statistical analyses are detailed within the specific individual chapters.

4 CHAPTER 4. The reliability of supra-patellar transverse sonographic assessment of femoral cartilage thickness in healthy adults

4.1 Abstract

PURPOSE: To determine intra-session reliability of femoral cartilage thickness measurements using ultrasonography and extend the pool of normative data for cartilage thickness measurements assessed by ultrasonography.

METHODS: 77 healthy participants (55 male and 22 female), with an average age of 43 ± 18 (mean ± SD) years volunteered. Resting supra-patellar ultrasound was used to image femoral cartilage thickness on two separate occasions a maximum of 7 days apart. Reliability was evaluated with intraclass correlation coefficient (ICC), Bland & Altman analysis, standard error of measurement (SEM and SEM%) and the smallest real difference (SRD and SRD%). Normative data was assessed using linear, multiple regression models and independent group t-tests.

RESULTS: The test-retest level of agreement at all locations was high (ICC 0.779 - 0.843), which increased to high-very high in young (ICC 0.884 - 0.920). The SEM% was 8.2 - 8.3% for all locations and reduced further to 5.4 - 6.3 % in younger. The SRD% was between 22.8 - 23.1% for the full sample and 14.9 - 17.5% in young only. Multiple regression analyses demonstrated that age, weight, female gender and a high physical activity frequency could significantly predict cartilage thickness at all locations ($P < 0.05$); however, female gender was the only significant independent predictor in all models (all $P < 0.01$). Females also had thinner cartilage at all locations ($P < 0.01$).

CONCLUSION: Supra-patellar ultrasonography demonstrates high intra-tester reliability and measurement precision and is a promising method to assess femoral cartilage thickness. Being female may impact femoral cartilage thickness more than other potential risk factors of knee osteoarthritis such as age, weight, and high physical activity frequency.

4.2 Introduction

In recent years US has been increasingly used to assess cartilage thickness. Axial supra-patellar US imaging is the most commonly documented technique (Akkaya et al. 2013b; Kara et al. 2013; Kaya et al. 2012; Malas et al. 2013; Özçakar et al. 2014; Öztürk et al. 2015). However, longitudinal US scanning of the knee has also been used previously (Tarhan et al. 2003; Yoon et al. 2008). Despite the emergence of US as a method to assess cartilage thickness, to date, only a few studies report validity (through the comparison of US measurements with cadaver specimens or MRI imaging) and reliability in relation to the sonographic evaluation of cartilage (Naredo et al. 2009; Tarhan et al. 2003; Yoon et al. 2008). Further knowledge of the accuracy and repeatability of this technique is essential if US is to be used as an effective tool to measure femoral cartilage thickness. Moreover, previous studies using US to measure femoral cartilage thickness have largely utilised a young adult sample (Malas et al. 2013; Özçakar et al. 2014), or has been confined to clinical populations (Kaya et al. 2012; Kara et al. 2013; Akkaya et al. 2013). There remains limited normative data for the sonographic measurement of femoral cartilage thickness in a large healthy adult sample.

As a result, the primary aim of this study was to assess the intra-session reliability of cartilage thickness measurements using sonography. Measurement precision was also assessed to identify the smallest change that can be considered actual change and not just a result of test-re-test error. A secondary aim of this study was to also extend the pool of normative healthy adult data for cartilage thickness measurements assessed by ultrasonography.

4.3 Methodology

4.3.1 Participants

Seventy-seven healthy volunteers (55 male and 22 female), with an average age of 43 ± 18 years, and with an average BMI of 24.9 ± 3.2, were enrolled. As outlined in Figure 4-1, several participants within this chapter were recruited from other experimental chapters.

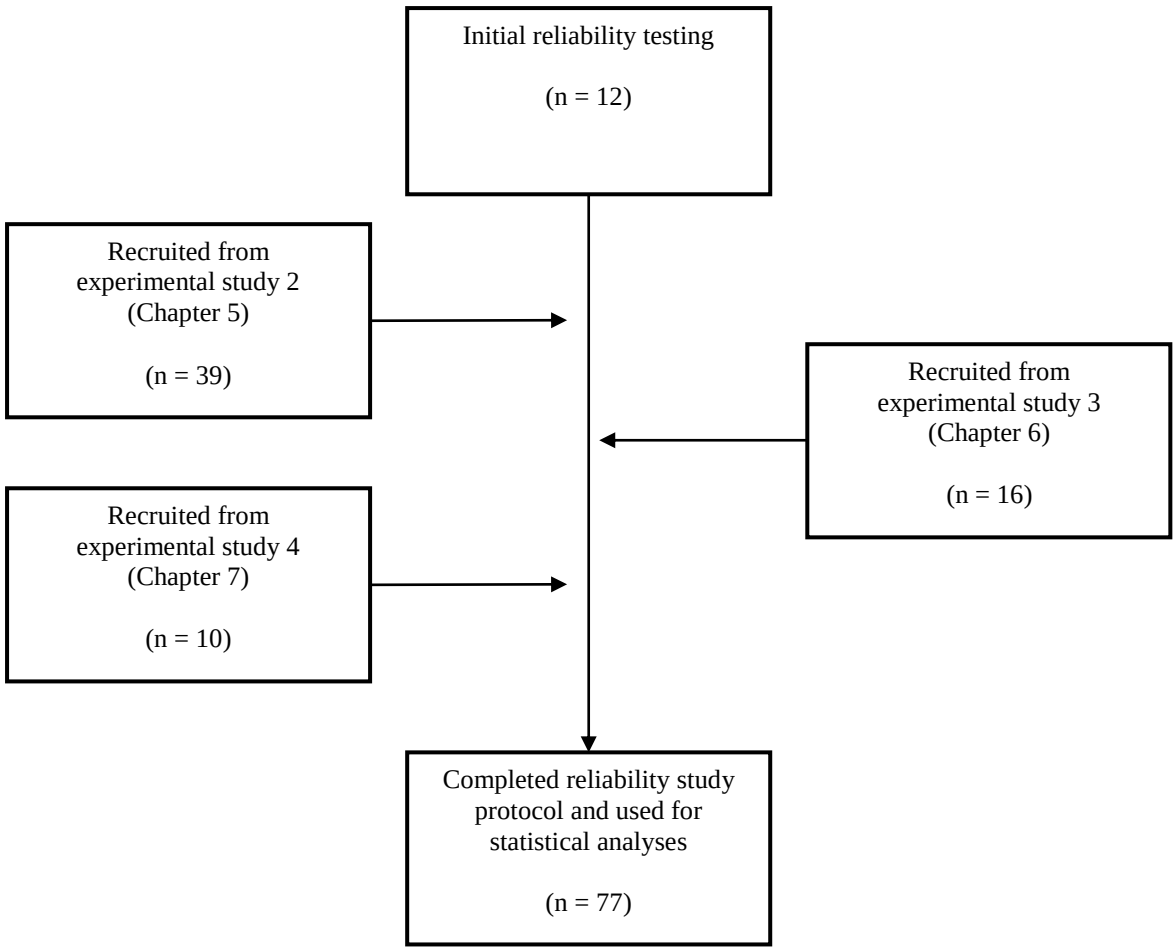


Figure 4-1 Participant flow diagram detailing participant recruitment

4.3.2 Experimental protocol

Participants were required to visit SSHES, Bangor University on two occasions with each session lasting approximately 60 minutes. During the initial visit participants completed a medical and basic physical activity questionnaire (Appendix C). Anthropometric measurement (body mass, height and BMI) were also completed (see Chapter 3). Subsequently, following a 15-30-minute period of seated rest ultrasonography was used to obtain images of the femoral articular cartilage as outlined in the general methodology (see Chapter 3). All participants completed their first and second visit at the same time of day and within a 7-day period. Participants were also asked to refrain from strenuous physical activity for 48 hours prior to each visit.

4.3.3 Statistical analysis

Reliability analysis

Agreement between measurements was evaluated using a one-way mixed, absolute agreement type, intraclass correlation coefficient (ICC) (Shrout and Fleiss, 1979). ICC values can be classified as low: 0.20–0.49; moderate: 0.50–0.69; high: 0.70–0.89; or very high: 0.90–1.00 (Munro, 2005). Paired t-tests together with Bland-Altman plots were used to provide an indication of systematic error (Atkinson and Nevill, 1998). The Bland-Altman plots demonstrate the mean difference between the cartilage thickness measurements at visit 2 and visit 1 (i.e. visit 2 minus visit 1) plotted against the mean of the two visits (i.e. visit 1 plus visit 2, divided by 2). These plots provide the reader with an opportunity to visualise the systemic variation and to determine whether heteroscedasticity is present within this data set. The standard error of the measurement (SEM) was calculated to establish measurement precision between visit 1 and 2. To do this, the following calculation was used:

$$(\text{SEM}) = \text{SD}\sqrt{1-\text{ICC}} \text{ (Thomas and Nelson, 1990)}$$

The SEM% was subsequently calculated using the following equation:

$$\text{SEM\%} = (\text{SEM}/\text{mean}) \times 100 \text{ (Beckerman et al. 2001)}$$

SEM% offers a measurement that is independent of the units of measurement, which provides an indication of the smallest value that represents a real change in a group of individuals. Thus, for cartilage thickness measurements to be considered different, the value must be outside this measurement error.

Furthermore, to calculate the smallest error in a single individual score, the smallest real difference (SRD) was calculated as follows:

$$\text{SRD} = 1.96 \times \text{SEM} \times \sqrt{2} \text{ (Eliasziw et al. 1994)}$$

The 1.96 value represents the confidence level, while the square root of 2 accounts for the measurement error on 2 tests. Finally, as per the SEM, the SRD was also expressed as a percentage value:

SRD% = (SRD / mean) * 100 (Beckerman et al. 2001)

All analyses were initially completed using the full dataset. Finally, split-group analysis was performed for each of the following groups: young (≤ 25 years of age), middle aged (26-50 years of age), and old age groups (≥ 51 years of age); males and females. This analysis provided an opportunity to determine whether age or sex of the participant influenced the level of intra-tester reliability and measurement precision.

Analysis of normative cartilage thickness data

Simple linear regression analyses were performed to determine the relationship between mean cartilage thickness of the right knee (at each location) and participant characteristics (age, body mass, height, and BMI). The left side was not used within the analysis of normative data as side to side differences in were found to be small and within measurement error in the present study. Moreover, others have reported no side dominance in cartilage thickness measurement (Eckstein et al. 2002) and have advocated the use of unilateral OA models in research (Dargel et al. 2009). Multiple linear regression models were used to explore the relationship between cartilage thickness (at each location) and potential risk factors; including age, BMI, and female gender and high frequency of weekly physical activity. Physical activity was considered 'high' when participants completed structured exercise training on a minimum of 5 days per week. In addition to multiple regression, mean cartilage thickness between sexes was also assessed by creating an equal sized (n = 17) sample matched for age and BMI. Independent t-tests were used to determine whether cartilage thickness differences existed between males and females at each measurement location (intercondyle notch, lateral condyle, medial condyle). For the multiple comparison of cartilage thickness between the three locations, Bonferroni corrections were used with $P < 0.016$ (0.05/3) for statistical significance. Finally, a one-way analysis of variance (ANOVA) was used to assess the differences in cartilage thickness measurements at each measurement location.

4.4 Results

The results from the US measurement of cartilage thickness and participant characteristics are displayed in Table 4-1. A total of 308 knees were scanned (right and left knee of 77

participants on two occasions). This produced a total of 1168 blinded images (77 participants were imaged three to four times per side on two occasions, i.e. visit 1 and visit 2). Cartilage thickness could be measured in most images. However, some individual images could not be measured as the hyperechoic line formed at the synovial-cartilage border and/or cartilage-bone border could not be clearly delineated. In total, the inability to confidently measure cartilage thickness accounted for 129 (11%), 180 (15%), and 221 (19%) of the available images for the intercondyle condyle, medial and lateral condyle, respectively. For the cartilage thickness reliability to be assessed a minimum of one image per location was required. Cartilage thickness could be measured in 306 knees (99.4%) at the medial condyle, 304 knees (98.7%) at the intercondyle notch, and 296 knees (96.1%) at the lateral condyle. Finally, a radar plot (Appendix D) provided evidence that that unclear images were more indicative of individuals who were older, male, and of a higher BMI.

Table 4-1 Physical characteristics of participants and knee cartilage thickness

	Men (n = 55)		Women (n = 22)		Total (n = 77)	
	Mean (SD)	**Range**	**Mean (SD)**	**Range**	**Mean (SD)**	**Range**
Age (years)	45 (18)	18 - 70	38 (20)	20 - 79	43 (18)	18- 79
Height (m)	1.77 (0.06)	1.64 - 1.95	1.67 (0.06)	1.51 - 1.78	1.74 (0.08)	1.5 – 1.95
Body mass (kg)	79.7 (11.1)	63.3 - 120.7	66.3 (11.6)	40.5 – 89.4	75.8 (12.7)	40.5 - 120.7
BMI	25.4 (3.0)	21.0-35.7	23.7 (3.5)	17.7 - 30.2	24.9 (3.2)	17.7 - 35.7
Knee cartilage thickness (mm)						
Right						
Lateral	1.93 (0.29)	1.43 - 2.73	1.71 (0.29)	1.23 - 2.37	1.87 (0.30)	1.23 - 2.73
Notch	2.20 (0.40)	1.28 – 3.22	1.75 (0.23)	1.27- 2.36	2.07 (0.43)	1.27 – 3.22
Medial	1.95 (0.38)	1.15 - 2.97	1.65 (0.28)	1.06 - 2.30	1.86 (0.38)	1.06 - 2.97
Left						
Lateral	1.79 (0.30)	1.02 - 2.37	1.68 (0.34)	0.93 – 2.44	1.76 (0.32)	0.93 - 2.44
Notch	2.17 (0.40)	1.44 - 3.12	1.84 (0.32)	1.18 - 2.46	2.08 (0.41)	1.18 - 3.12
Medial	1.84 (0.34)	1.01 - 2.60	1.63 (0.31)	1.08 – 2.28	1.78 (0.34)	1.01 - 2.60

4.4.1 Reliability analysis

The ICC and 95% confidence intervals (95% CI) for the data are shown in Table 4-2. The ICC's indicate that the level of agreement at all locations was high (ICC between 0.779 – 0.843), with the highest at the intercondyle notch, followed by the medial condyle and then the lateral condyle. Subsequent analyses revealed that the level of agreement between measurements was considerably improved when considering younger participants (≤ 25 years of age) only (Table 4.3). In addition, the image quality and clarity was typically better in younger individuals as highlighted in Figure 4-2. Results also demonstrated that the intra-tester reliability of cartilage thickness measurements was generally similar when male and female groups were analysed separately (Table 4.3).

Table 4-2 Reliability of cartilage thickness measurements made at visit 1 and visit 2 for all locations

Location	ICC (95% CI)	SEM	SEM%	SRD	SRD%
Notch	0.843 (0.790 - 0.883)	0.17	8.2	0.47	22.9
Medial	0.834 (0.778 - 0.876)	0.15	8.2	0.42	22.8
Lateral	0.779 (0.707 - 0.835)	0.15	8.3	0.42	23.1

ICC = intra-class correlation; CI = confidence intervals; SEM = standard error of measurement; SRD = smallest real difference

Table 4-3 Reliability of cartilage thickness measurements made at visit 1 and visit 2 for all locations with comparisons between age and gender

Location	ICC (95% CI)	SEM	SEM%	SRD	SRD%
Young					
Notch	0.920 (0.854 - 0.957)	0.12	5.7	0.32	15.8
Medial	0.884 (0.792 - 0.937)	0.11	6.3	0.32	17.5
Lateral	0.906 (0.830 - 0.949)	0.10	5.4	0.28	14.9
Middle aged					
Notch	0.843 (0.747 - 0.905)	0.18	8.4	0.49	23.3
Medial	0.800 (0.684 - 0.877)	0.13	6.8	0.35	18.9
Lateral	0.639 (0.453 - 0.772)	0.17	9.2	0.47	25.4
Old					
Notch	0.779 (0.651 - 0.864)	0.19	9.2	0.51	25.4
Medial	0.832 (0.731 - 0.898)	0.17	9.6	0.48	26.5
Lateral	0.788 (0.661 - 0.872)	0.15	8.7	0.42	24.2
Male only					
Notch	0.804 (0.725 - 0.862)	0.18	8.3	0.50	23.3
Medial	0.803 (0.725 - 0.861)	0.16	8.7	0.45	24
Lateral	0.744 (0.645 - 0.819)	0.15	8.2	0.42	22.8
Female only					
Notch	0.838 (0.723 - 0.908)	0.12	6.9	0.34	19.0
Medial	0.870(0.775 - 0.927)	0.10	5.8	0.27	16.2
Lateral	0.828 (0.708 - 0.902)	0.12	6.8	0.32	19.0

ICC = intra-class correlation; CI = confidence intervals; SEM = standard error of measurement; SRD = smallest real difference); young (≤ 25 years of age), middle aged (26-50 years of age), and old age groups (≥ 51 years of age)

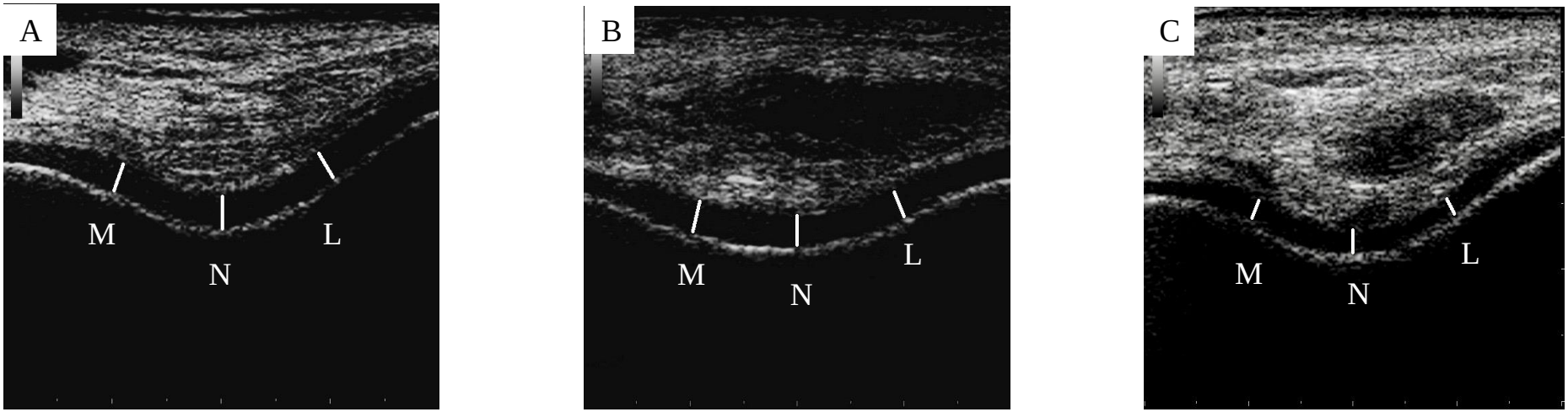


Figure 4-2 US transverse image of the femoral articular cartilage demonstrating the difference in image quality and clarity between young, middle aged, and old groups. Image A) represents the 'young' group (23-year-old male), image B) represents the 'middle-aged' group (44-year-old male), and image C) represent the 'old' group (69-year-old male). M = the location of medial condyle; N = the intercondyle notch; L = the lateral condyle

Systemic variation in cartilage thickness measurements at the notch, medial condyle and lateral condyle are shown by the Bland-Altman plots (Figure 4-3). The plots suggest that slightly higher variation (i.e. heteroscedasticity) may exist for higher cartilage thickness measurements, particularly at the notch and medial condyles. Moreover, results of the paired t-tests showed no significant difference in cartilage thickness between visit 1 and visit 2 for the medial (1.83 vs 1.82 mm; P = 0.760) and lateral condyle locations (1.81 vs 1.81 mm; P = 0.860). However, at the intercondyle notch, a small but significantly greater cartilage thickness measurement was obtained at visit 2, thus indicating that measurements made during the second visit may be systematically higher compared to measurements made at visit 1 (2.03 vs 2.08 mm; P = 0.016). When data was split based on age of the individuals, paired t-tests between visit 1 and visit 2 did not reveal any systematic differences in measurements made in young participants. Furthermore, although mean differences in cartilage thickness measurements tended to be slightly higher in middle aged and older participants at most measurement locations compared to the young group, a significant difference between visit 1 and visit 2 was only present at the intercondyle notch in the middle-aged participants.

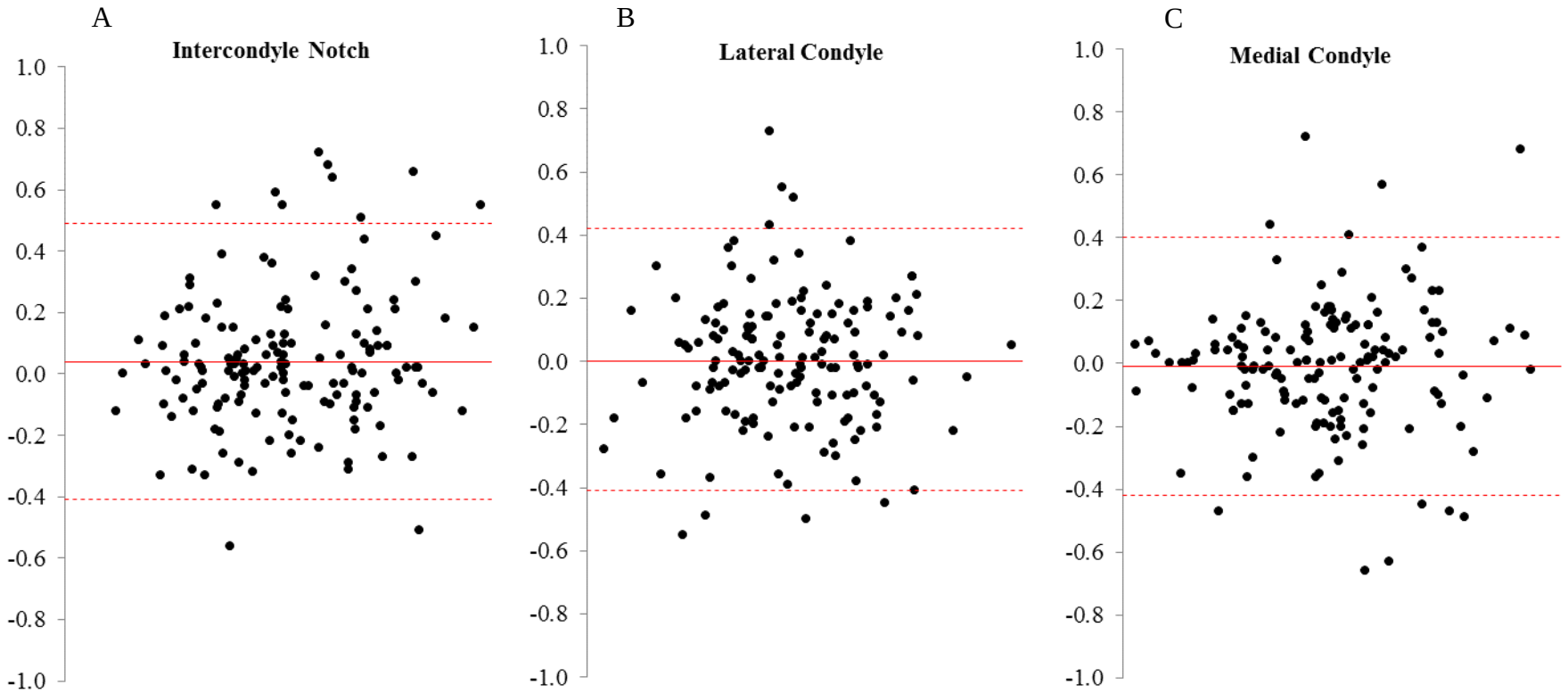


Figure 4-3 The Bland-Altman plots demonstrate the mean difference between the cartilage thickness measurements at visit 2 and visit 1 (i.e. visit 2 minus visit 1) plotted against the mean of the two visits (i.e. visit 1 plus visit 2, divided by 2). Plot A) represents intercondyle notch, B), lateral condyle and C) medial condyle (solid line represents mean difference and dashed lines represent upper and lower limits of agreement)

4.4.2 Measurement precision

The SEM is provided for each location in Table 4.2. The value ranged from 0.15 - 0.17 mm for all locations. Interestingly, and in agreement with the intra-class correlation analysis, the SEM was lowest in the split group analysis of young participants only (Table 4.3). Moreover, the SEM%, which provides a measure independent of units, indicates that differences in groups of individuals above 8.2-8.3% can be considered a real change and not difference associated with measurement error. Overall, the SEM% values for all analyses (Table 4.2 and 4.3) provide evidence of a relatively low range (5.4 – 9.6%). Moreover, the smallest real change is the measurement error in a single individual cartilage thickness. Table 4.2 demonstrate that the SRD is between 0.42 and 0.47 mm for all locations. In relative terms, this equals 22.8 – 23.1%. An improvement in the smallest real change was shown in young participants (0.28 - 0.32 mm) and when analysing females only (0.27 - 0.34 mm).

4.4.3 Sonographic assessment of cartilage thickness

Femoral cartilage thickness did not differ between the right and left intercondyle notch, or the right and left medial condyle. Although differences were observed at the lateral condyle between the left and right knee (1.78 vs 1.88 mm, P = 0.04), the difference was small (5.6%) and within the SEM. For this reason (and as previously stated in the methodology), normative data analyses were based on the right side only.

Age was found to have a negative relationship with lateral cartilage thickness in men (Figure 4-5A). Participant weight was found to have a positive relationship with cartilage thickness at the intercondyle notch (Figure 4-4C). Interestingly, when females and men were assessed separately, this positive correlation was only found in men (Figure 4-5C). Participant height was also found to have a positive relationship with cartilage thickness at all locations (Figure 4-4B); however, when analysis was separated for males and females, a positive relationship remained between height and lateral condyle cartilage thickness in males only. Moreover, a negative relationship between height and intercondyle notch thickness was found in females. In addition, BMI was found to have a negative relationship with lateral and medial condyle thickness in men, but not women (Figure 4-5D). The correlation coefficient, levels of significance and regression equation are presented in Figure 4-4 for the full dataset, and in Figure 4-5 for the comparison between males and females.

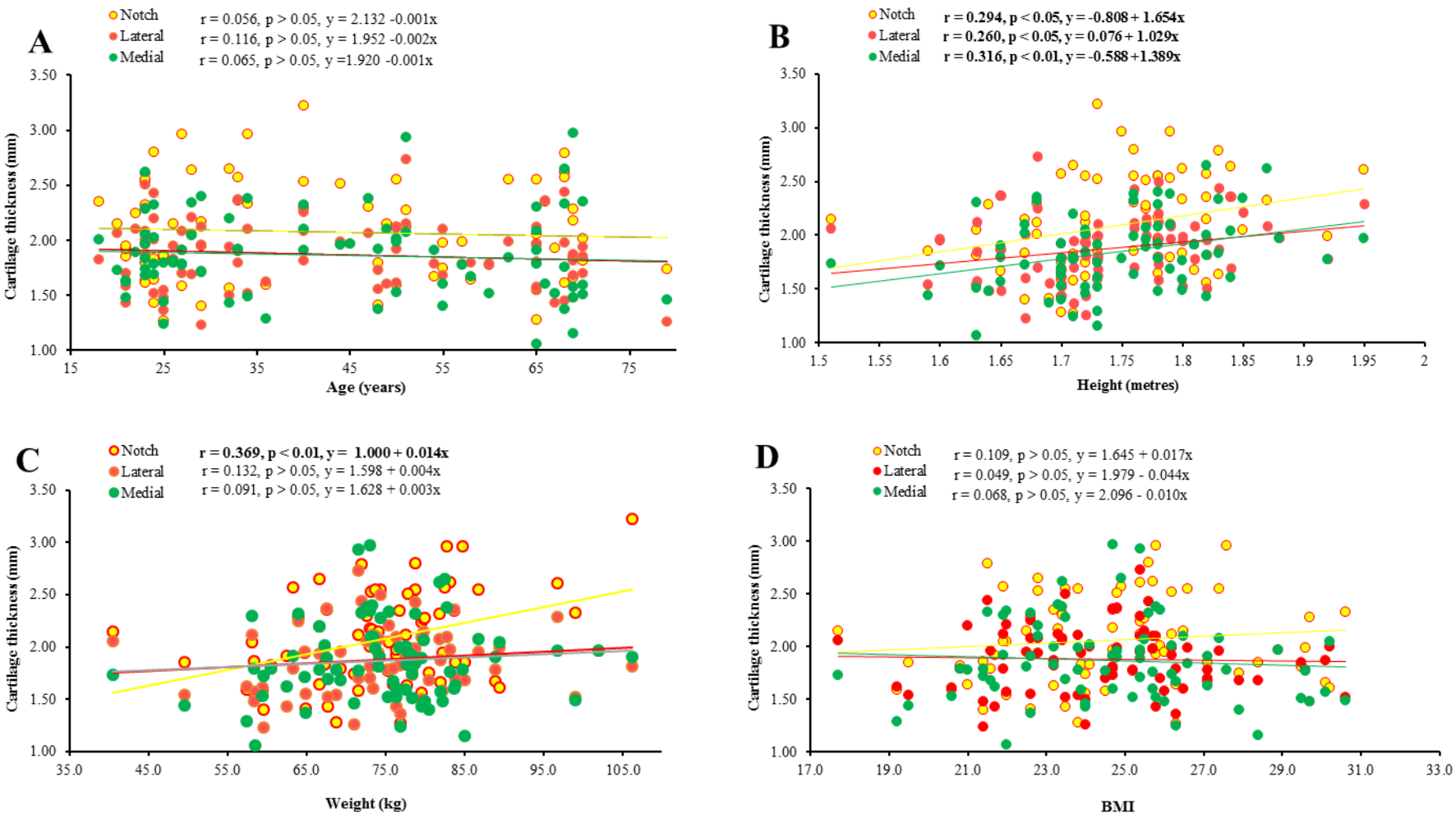


Figure 4-4 Variation of mean femoral cartilage thickness at the intercondyle notch, lateral condyle and medial condyle with physical characteristics of the participants. A) age, B) height, C) weight, and D) BMI. R-value, significance value and regression equation are also presented above with significant findings highlighted in bold. Yellow, red and green trend line = intercondyle notch, lateral condyle and medial condyle, respectively

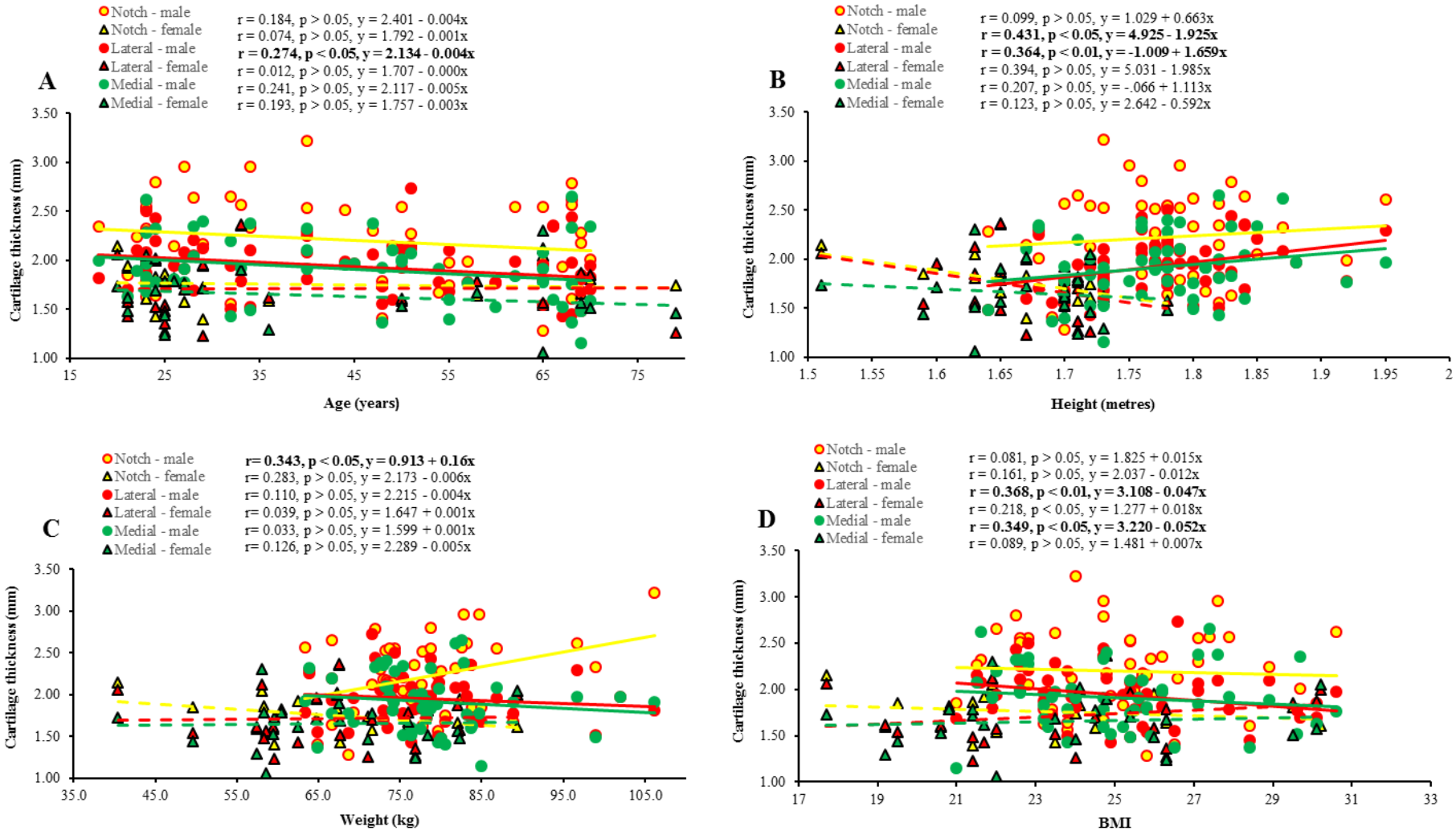


Figure 4-5 Presents variation of mean femoral cartilage thickness at the intercondyle notch, lateral condyle and medial condyle with physical characteristics of the participants for both males and females A) age, B) height, C) weight, and D) BMI. R-value, significance value and regression equation are also presented above with significance highlighted in bold. Solid trend line and circular data points = male; medium dashed trend line and triangles = female. Yellow = notch; red = lateral; green = medial

Age, weight, female gender and a high physical activity frequency (> 5 sessions per week) were the independent variables included in the multiple regression model (Table 4-4). The model could predict 28.8% of the variance in cartilage thickness at the intercondyle notch ($P < 0.01$). However, gender was the only independent variable to significantly contribute to the model. The beta coefficient indicates that in this sample the cartilage thickness in females was 0.38 mm lower than males ($P < 0.01$). Age was the second biggest contributor to the model ($P = 0.08$). For the lateral condyle, the regression model could predict 16% of the variance in cartilage thickness ($P < 0.05$). As per the previous regression model, gender was the only independent variable to significantly contribute to the model ($P = 0.008$). Finally, age, weight, female gender and a high physical activity frequency at the medial condyle could significantly predict 15.1% of the variance in cartilage thickness at the medial condyle ($P < 0.05$). However, again, gender was the only independent variable that contributed to the model ($P = 0.03$).

Analysis of the stable model, i.e. with sex (a dummy variable) as the only predictor variable the following models were produced: For the femoral intercondyle notch, femoral cartilage thickness in females could be calculated as $2.204 + (-0.450 \times 1) = 1.754$ mm, and in males it could be calculated as $2.204 + (-0.450 \times 0) = 2.204$ mm. The model was significant ($p < 0.001$). For the femoral lateral condyle, femoral cartilage thickness in females could be calculated as $1.933 + (-0.219 \times 1) = 1.714$ mm, and in males it could be calculated as $1.933 + (-0.219 \times 0) = 1.933$ mm. This model was also significant ($p < 0.01$). Finally, for the femoral medial condyle, femoral cartilage thickness in females could be calculated as $1.946 + (-0.295 \times 1) = 1.651$ mm, and in males it could be calculated as $1.946 + (-0.295 \times 0) = 1.946$ mm. This model was also significant ($p < 0.01$).

Table 4-4 The relative important and statistical significance of potential risk factors; including age, weight, female gender and high frequency of weekly physical activity on cartilage thickness measured by ultrasonography at three locations

Notch			
Independent Variable	**Regression coefficient**	**T**	**P-value**
Intercept	1.624	3.750	P < 0.001
Age	-0.003	-1.212	0.230
Weight	0.010	1.786	0.079
Female gender	-0.367	-3.016	**0.002***
Exercise habits (> 5 x / wk)	-0.071	-0.581	0.563
Lateral condyle			
Independent Variable	**Regression coefficient**	**T**	**P-value**
Intercept	2.166	7.086	P < 0.05
Age	-0.002	-1.149	0.255
Weight	-0.002	-0.506	0.615
Female gender	-0.253	-2.755	**0.008***
Exercise habits (> 5 x / wk)	0.112	1.205	0.233
Medial condyle			
Independent Variable	**Regression coefficient**	**T**	**P-value**
Intercept	2.235	6.114	P < 0.05
Age	-0.002	-0.855	0.396
Weight	-0.004	-0.843	0.402
Female gender	-0.355	-3.071	**0.003***
Exercise habits (> 5 x / wk)	0.060	0.509	0.613
*represents significance contribution to model (P < 0.05)			

Results demonstrated that cartilage thickness was thicker at the intercondyle notch compared to the medial and lateral condyle (Figure 4-6). However, there was no difference in cartilage thickness between the medial and lateral condyle ($P > 0.05$).

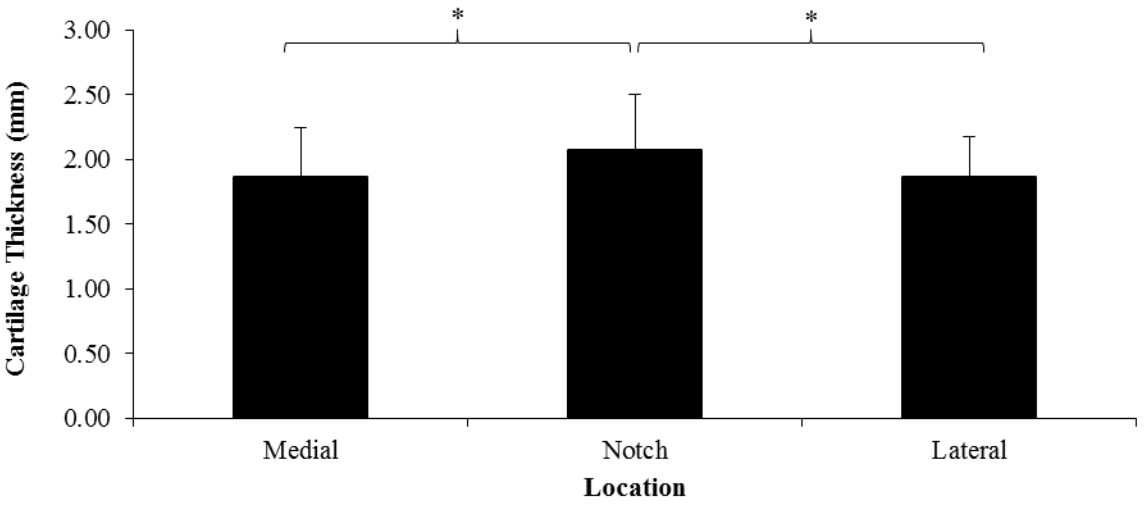


Figure 4-6 Mean cartilage thickness measurements at the medial condyle, intercondyle notch and the lateral condyle: * = significant difference between groups at $P < 0.01$ level. Data are means ± SD

To further assess for differences in mean cartilage thickness between sexes an equal sized (n = 17) sample matched for age and BMI was created. Results demonstrated that mean cartilage thickness was lower in females at the intercondyle notch, lateral condyle and medial condyle (Figure 4-7) than that of the matched male group. The biggest difference in mean cartilage thickness between males and females was at the medial condyle (2.00 mm versus 1.60 mm, respectively).

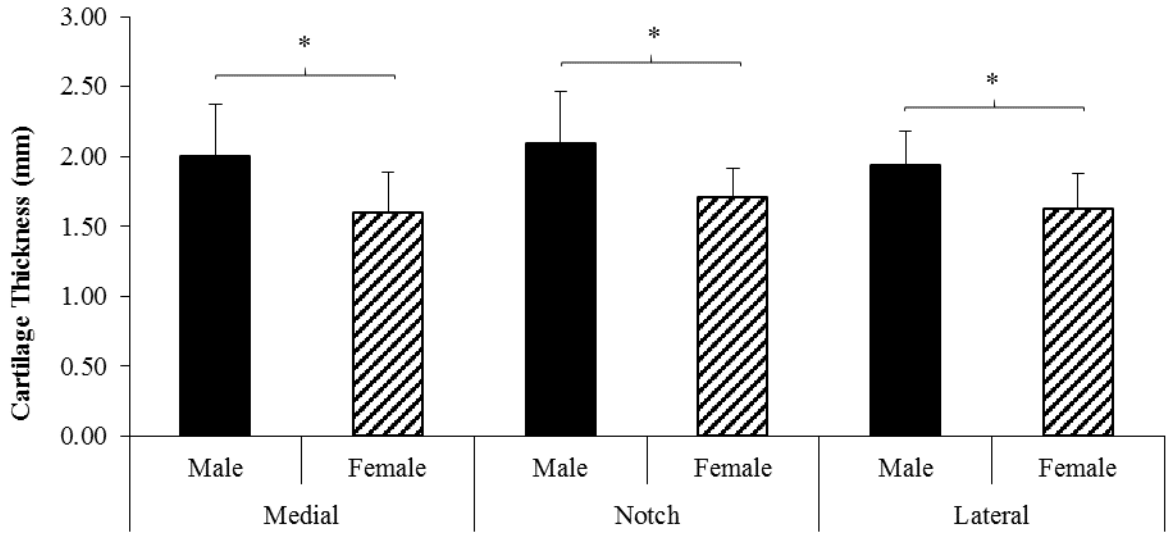


Figure 4-7 Mean cartilage thickness measurements at the medial condyle, intercondyle notch and the lateral condyle for both male (n = 17) and female (n = 17) participants, matched for age and BMI. * = significant difference between groups at $P < 0.01$ level. Data are means ± SD.

4.5 Discussion

The use of supra-patellar transverse sonography to assess femoral cartilage thickness is a novel technique, which required further study into its reliability and accuracy. The purpose of the present study was to ascertain the intra-tester reliability of supra-patellar transverse US of femoral cartilage thickness in a group of healthy males and females across a wide range of ages. Notably, the present study provides evidence of high intra-tester reliability for femoral cartilage thickness at the intercondyle notch, medial condyle, and lateral condyle, as well as a reasonably small measurement error. Additional analysis revealed that both intra-tester reliability and measurement precision reliability was better in young healthy individuals when compared with older counterparts.

Importantly, this study found that in healthy individuals, supra-patellar transverse ultrasonography allowed a quick and straightforward assessment of femoral cartilage. The high ICCs found in the present study [intercondyle notch 0.843 (0.790 - 0.883), medial condyle 0.834 (0.778 - 0.835) and lateral condyle 0.779 (0.707 - 0.876)], are comparable to

previously reported ICCs using a very similar standardised protocol in a small sample of flexed cadaver knee (age of death was 76-89 years) (Naredo et al. 2009). Interestingly, in both studies the level of agreement at the lateral condyle was lower compared to the intercondyle notch. One possibility is that the lateral and medial condyles are prone to an increased level error related to the inclination and positioning of the US transducer (Naredo et al. 2009). This is supported by previous evidence using MRI, which reported that central weight regions of femoral condyles often provide greater accuracy than boundary areas (Koo et al. 2005). Results in the present study also revealed that intra-tester reliability was considerably greater in younger individuals compared to middle-aged and older individuals. Given that limited, if any, degenerative change would be expected in young healthy individuals, the increased reliability in young individuals might be due to the femoral cartilage appearing considerably clearer in young participants. In contrast, image quality in older individuals was often lower, thus reducing the ability of the investigator to delineate images and offer such precise measurements.

Images obtained within this study provided a clear hyperechoic line formed at the synovial-cartilage border and/or cartilage-bone border that allowed femoral cartilage thickness to be assessed in most but not all cases. Compared to the study by Yoon and colleagues (2008), cartilage thickness could be measured in a greater proportion of knees at the medial condyle (98.7 vs 70.6%) and lateral condyle (96.1 vs 90.1%) in the current study. Differences in the ability to measure cartilage thickness between the two studies are likely to relate to the participants (i.e. OA vs healthy individuals in the current study). Several degenerative changes, including, roughened and fibrillated articular cartilage, cartilage loss, asymmetrical narrow, as well as abnormalities at the subchondral bone have previously been associated with poorly defined hyperechoic cartilage borders (Yoon et al. 2008; Özçakar et al. 2014). Moreover, despite great care being used to standardise the US assessment of the knee and to replicate the positioning of both the participant and transducer between sessions, other factors such as poor transducer positioning or movement artefact, may also contribute to poor image quality (Naredo et al. 2009). Furthermore, in the current study, of the 7 knees which could not be measured, individuals were all male, mostly older and had a higher BMI. These factors and the relationship with the ability to measure cartilage thickness using US warrant further investigation.

Femoral cartilage thickness measurements in the present study were comparable to several previous studies using the same US methodology in similarly aged healthy individuals (Kaya et al. 2012; Malas et al. 2014). In contrast, others have reported slightly greater femoral cartilage thicknesses in young (25–40 years) healthy individuals compared to the present study (Özçakar et al. 2014). The present study also found a significantly thicker cartilage thickness at the intercondyle notch compared to the lateral and medial condyle. This difference has not been observed to the same extent in several other studies (Kaya et al. 2012; Malas et al. 2014; Özçakar et al. 2014) and may be related to differences in biomechanical loading.

In addition, femoral cartilage thickness did not differ between the right and left intercondyle notch, or the right and left medial condyle. Although differences were observed at the lateral condyle between the left and right knee (1.78 vs 1.88 mm, P = 0.04), the difference was small (5.6%) and within the SEM. Side to side differences in thickness have previously been reported; however, differences in cartilage thickness tend to be small (total knee joint: 3.8 ± 3.1%) with no significant differences for limb dominance (Eckstein et al. 2002). Moreover, additional research has previously reported good correlations between morphological dimensions of the left and right side and has advocated the use of unilateral OA models in research (Dargel et al. 2009).

The current study also found females had lower cartilage thickness at all locations compared to males and is consistent with previous studies using both MRI and US (Faber et al. 2001; Otterness & Eckstein 2007; Özçakar et al. 2014). Furthermore, regression analyses in the present study, found that female gender was the only variable that could explain the variation in cartilage thickness. The lower femoral cartilage thicknesses observed in the present study may relate to differences in body size between men and women. This is supported by the current finding that women have thinner cartilage thickness compared to men after the adjustment for age and BMI (Figure 4-7), and previously, after adjustment for body height and weight (Otterness and Eckstein, 2007). Differences between males and females may also relate to differences in the sex hormone oestrogen (Ben-Hur et al. 1997), which is understood to act upon oestrogen receptors found in articular cartilage (Ushiyama et al. 1999), and/or to differences in the dynamic loading across the knee joint between men and women (Cicuttini et al. 1999).

Further analyses demonstrated that age was negatively associated with lateral cartilage thickness, but only in males. Similarly, several studies have previously found ageing to be negatively associated with femoral cartilage thickness assessed by both US (Özçakar et al. 2014) and MRI (Hudelmaier et al. 2001). The results of the current study suggest that the lateral femoral condyle might be the most prominent site for age related change. Furthermore, although age has previously been found to be negatively associated with femoral cartilage thickness in both men and women (Özçakar et al. 2014), the present study suggests that men are more at risk of age related change in lateral condyle cartilage thickness. This finding is particularly surprising given that older women are at increased risk of OA (Felson et al. 1987) and may relate to the small sample of females in the present study.

Anthropometric variables such as body height and body weight may also influence femoral cartilage thickness. In the current study, a positive relationship was found between body height and cartilage thickness for all three locations. Several previous studies have also found body height to be positively associated, albeit weakly, with cartilage thickness (Otterness & Eckstein 2007; Hudelmaier et al. 2001). In contrast to body height, a positive relationship between body weight and cartilage thickness was only apparent at the intercondyle notch. Other studies have failed to find a relationship between body weight and cartilage thickness (Blazek et al. 2014). However, when the relationship was explored separately for males and females in the current study, body weight and body height demonstrated a different relationship with femoral cartilage thickness. Both body height and body weight were shown to have a positive relationship with cartilage thickness at various locations in men, while in females, body weight was unrelated to femoral cartilage thickness and body height was negatively related at certain locations. Similarly, Hudelmaier et al. (2003) have also demonstrated that neither body weight or height were correlated with femoral cartilage thickness in women, and only body height was positively correlated with femoral cartilage thickness in men. Reasons for the difference between men and women are unknown. However, it appears that the higher joint loads that are related to body size may have a more favourable impact on cartilage thickness of healthy men compared to women. Whether the relationship extends to a group of men with a greater variation in body size remains unclear. The present study also found that BMI had a negative relationship with both lateral and medial condyle thickness in men. This supports previous research indicating having a high BMI may increase the risk of reduced cartilage thickness and knee OA (Reijman et al. 2007). The results of the current study would suggest that while being either heavier or taller may be

positive for cartilage thickness in men, an unfavourable body composition may reduce cartilage thickness. This may also suggest that muscle function and physical fitness may have a key role in cartilage thickness morphology. Although exercise frequency as a measure of physical activity level was not associated with cartilage thickness in the present study, future research, together with more refined measures of physical activity is required to explore the potential relationship and determine whether a moderation effect exists.

A primary limitation of this study was the inability to determine the validity of femoral cartilage thickness measurements made using US with a gold standard such as MRI. Nonetheless, US may be regarded as a promising measurement technique that has demonstrated a good agreement in both cartilage thickness measurements made using US and MRI (Tarhan et al. 2003) as well as US and anatomical specimens (Naredo et al. 2009). Nevertheless, this level of agreement is not a universal finding, particularly when using supra-patellar axial US to assess medial condyle thickness (Yoon et al. 2008) and when severely damaged knees are included in the analysis (Naredo et al. 2009). Importantly, caution is warranted when considering the validity and reliability of sonographic measures of cartilage thickness when the sample includes older individuals, and individuals with significant knee OA. In addition, unlike the analysis of MRI, US cartilage thickness measurements are largely limited to the femoral plate and do not offer the ability to assess other morphological measurements such as cartilage volume. It also remains unknown whether US is sensitive to minor changes or acute changes following acute loading. A further limitation of the present study relates to the fact that inter-tester reliability was not assessed. This is particularly important given the usefulness of sonographic cartilage thickness measurements as a clinical and research tool relies on the ability to make direct comparisons between studies.

4.6 Conclusion

This small cross-sectional study of the healthy adults, which includes a wide range of ages, provides evidence of high intra-tester reliability for all femoral cartilage locations (ICC's between 0.779-0.843) and measurement precision (SEM% between 8.2-8.3%), which is better in younger participants (ICC's between 0.884-0.920 and SEM% 5.4-6.3%). In young participants, differences between groups or following an intervention that are greater than 6.3% represent a real difference and not just measurement error. Finally, this study also

extends the pool of normative data. The current study also provides clear evidence that considerable variability exists in the femoral cartilage thicknesses of healthy individuals. Cartilage thickness appears greatest at the intercondyle notch compared to the medial and lateral condyles. Furthermore, there is also evidence to suggest that females have reduced cartilage thickness compared with males and that both ageing and anthropometric measures affect cartilage thickness differently in males and females.

Study contributors:

Harry Roberts, A/Prof Jeanette Thom and Dr Jonathan Moore conceived and designed study; Harry Roberts, Claire Griffiths-McGeever and Lewis Angell collected raw data; Harry Roberts completed all data analyses and prepared chapter.

5 CHAPTER 5. A pilot study to assess the effect of ageing and physical activity on serum biomarkers and cartilage thickness

5.1 Abstract

PURPOSE: To investigate the effect of age and physical activity on serum biomarker concentrations and femoral knee cartilage thickness in a cross-sectional sample of healthy males.

BACKGROUND: This study utilizes several emerging biomarkers that have been associated with early degenerative changes, including serum COMP, HA and lubricin.

METHODS: A cross-sectional heterogeneous sample of 81 healthy males (age: mean (range): 43 (18–70) years; body mass: 79.4 (63.3-106.2) kg; VO_2max: 44.6 (24-67) $mL \cdot kg \cdot min^{-1}$) were investigated. Venous blood samples and supra-patellar US imaging scans were obtained following 30 min of seated rest. Serum COMP, HA and lubricin concentrations were determined via commercially available ELISA. Physical activity level and VO_2max were assessed using questionnaires and via an incremental bike test, respectively. Primary statistical analyses were performed using linear, hierarchical, and moderated multiple regression models.

RESULTS: Ageing was positively associated with serum COMP ($r = 0.299$; $P < 0.01$) and negatively associated with lateral condyle femoral cartilage thickness ($r = 0.368$; $P < 0.01$). Following adjustment for body weight, injury, 7-day physical activity, and 12-month physical activity, only the negative association with lateral condyle femoral cartilage thickness remained. Ageing was not associated with serum HA, lubricin or femoral cartilage thickness at either the medial or intercondyle notch. The age-related decline in lateral condyle cartilage thickness was not moderated by physical activity levels over the last 12 months, 7 days or by cardio-respiratory fitness level (all $P > 0.05$).

CONCLUSION: In this healthy male sample, ageing was not associated with changes in serum biomarkers. However, ageing was associated with a reduction in cartilage thickness at the lateral condyle, possibly due to an age-related change in the distribution of joint load across the joint. In contrast to the hypothesis, physical activity does not appear to moderate the negative association between lateral condyle thickness and age. Overall, physical activity appears to be well tolerated. Moreover, higher levels of physical activity over the last 12 months may have a positive effect on lateral condyle cartilage thickness in this cohort.

5.2 Introduction

With age, articular cartilage undergoes several changes that may increase the vulnerability of cartilage to degenerative change (Martin and Buckwalter, 2002) and reduce its ability to overcome the effects of mechanical stress and injury (Luria and Chu, 2013). Several studies using MRI have indicated that ageing is associated with surface fibrillation of articular cartilage (Arokoski et al. 2000; Pap et al. 1998; Young et al. 2006), an increased number of defects (Ding et al. 2005), and a reduction in cartilage volume and thickness (Ding et al. 2005; Hanna et al. 2005; Hudelmaier et al. 2001; Mosher et al. 2010). However, limited research has assessed whether femoral cartilage thickness is reduced with healthy ageing. Furthermore, the weight bearing sites of the femur are also associated with the earliest changes in thickness (Karvonen et al. 1994), thus providing a key measure of cartilage morphology. Moreover, studies investigating femoral cartilage thickness have largely focused on clinical populations (Akkaya et al. 2013a; Kara et al. 2013; Kaya et al. 2012). Ageing and the association with femoral cartilage thickness will be studied using axial supra patellar ultrasonography, which has been shown to be a reliable technique to assess femoral cartilage (see Chapter 4).

In addition to potential morphological alterations of articular cartilage, ageing may be associated with further cellular and extra-cellular matrix changes (Martin and Buckwalter, 2002). Several molecules or molecular fragments from the extracellular matrix, or cellular metabolism of the articular cartilage, subchondral bone and synovial issue, have been recognised as indicators of normal biological processes and pathogenic processes. Biomarkers of interest in the present study are serum COMP, HA, and lubricin; these markers are understood to predominantly reflect cartilage degradation/turnover, synovial tissue turnover, and joint lubrication, respectively (see Chapter 2). Therefore, it is important to determine whether healthy ageing influences morphological properties of cartilage, as well as novel serum biomarkers that are associated with degenerative change.

OA results from a combination of several factors (see Chapter 2), while the potential impact of ageing on OA may be influenced by other variables, including physical activity. Physical activity is used as both a preventative and therapeutic measure for OA (Leong and Sun, 2014) and has been demonstrated to have a positive effect on several other known OA risk factors, including, BMI, muscle strength, and cardiovascular fitness (Beckwée et al. 2013). Although physical activity is understood to generally have a positive effect on the knee joint

(Bosomworth, 2009; Cymet and Sinkov, 2006; Esser and Bailey, 2011; Urquhart et al. 2011), the relationship with femoral cartilage thickness and biochemical indices of the knee joint is unclear. Currently there is no clear evidence to suggest that physical activity history or chronic exercise training results in a positive or negative change in cartilage volume or thickness at the knee or influences serum biomarker profile. Furthermore, the benefits of physical activity may depend upon the type (Driban et al. 2015; Spector et al. 1996a), and the intensity of physical activity (Franciozi et al. 2013; Vignon et al. 2006).

Therefore, **t**he primary aim of this study was to explore a heterogeneous sample to determine the effect of age on femoral cartilage thickness and serum biomarkers in healthy individuals. The secondary study aim was to determine whether any potential reduction in femoral cartilage thickness and/or serum biomarkers with age is moderated by physical activity history and training status. Exploratory analyses were also completed to investigate whether other factors influence an individual's femoral cartilage thickness and/or serum biomarker profile, including: body weight, BMI, joint load and injury.

Hypothesis 1: Healthy ageing is associated with an increase in serum COMP and HA concentration and a reduction in both serum lubricin and cartilage thickness

Specific objective 1: To test this hypothesis, a resting baseline blood sample was obtained via venepuncture and serum COMP, HA and lubricin concentrations were determined using commercial ELISA. Resting femoral cartilage thickness was obtained using US. The relationship between age and biomarker concentration and femoral cartilage thickness were assessed.

Hypothesis 2: Engaging in physical activity has a positive effect of biochemical indices and cartilage thickness, ameliorating the age related degenerative change

Specific objective 2: To test this hypothesis, an individual's physical activity level was assessed by objective measurement and two questionnaires (over last 7 days and last 12 months). These measures were subsequently included as an independent variable to determine whether the relationship between age and serum biomarkers, and age and femoral cartilage thickness, are confounded by physical activity.

5.3 Methodology

5.3.1 Participants

A heterogeneous sample of eighty-one healthy men was recruited to this study (Figure 5-1). Participants were required to be healthy, male, aged between 18 – 75 years, with a BMI of < 30 kg/m^2.

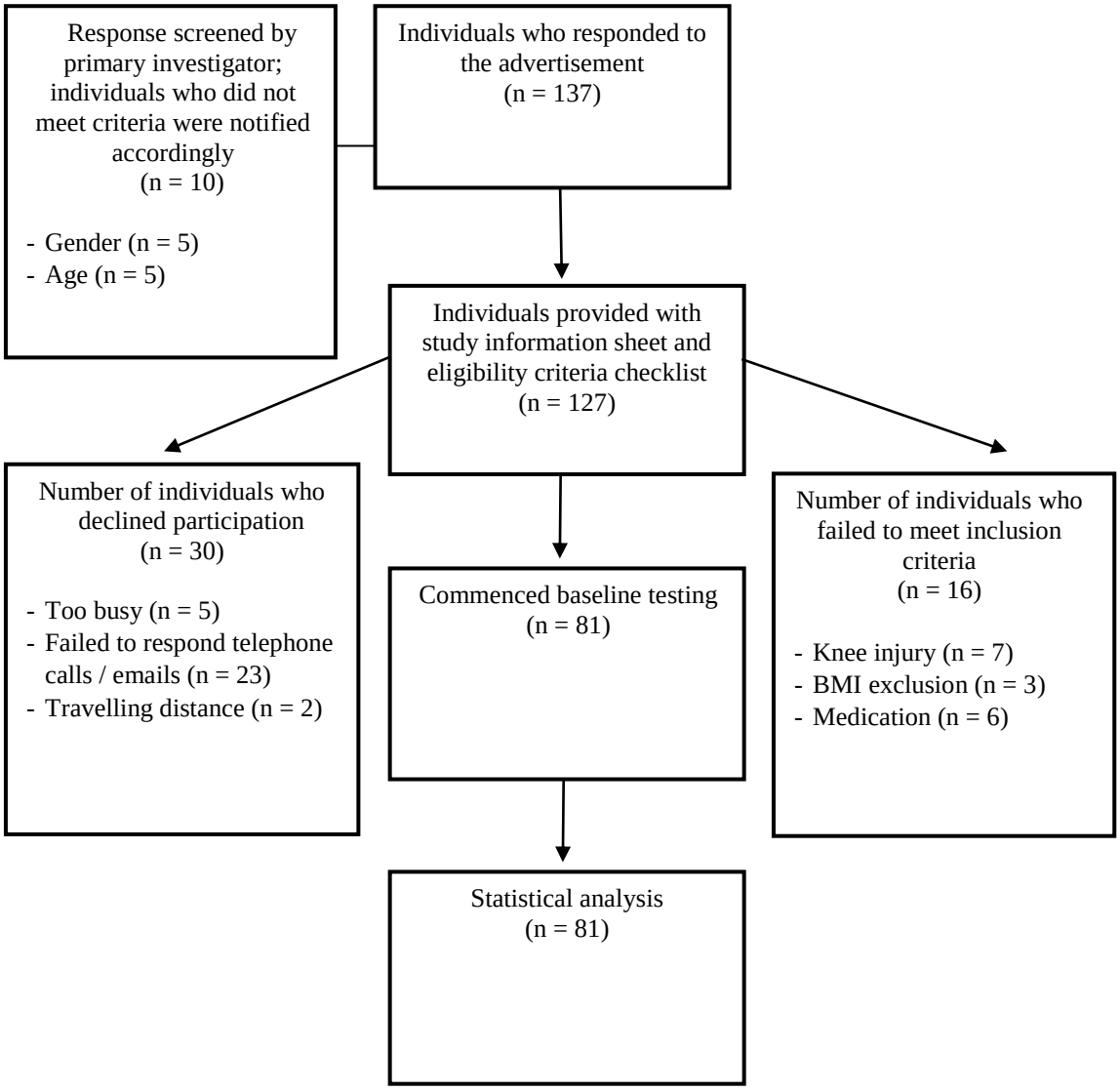


Figure 5-1 Participant flow diagram detailing participant recruitment, baseline testing and protocol completion

5.3.2 Experimental protocol

In this cross-sectional, correlational study, participants were required to visit SSHES, Bangor University on two separate occasions as outlined below in Figure 5.1.

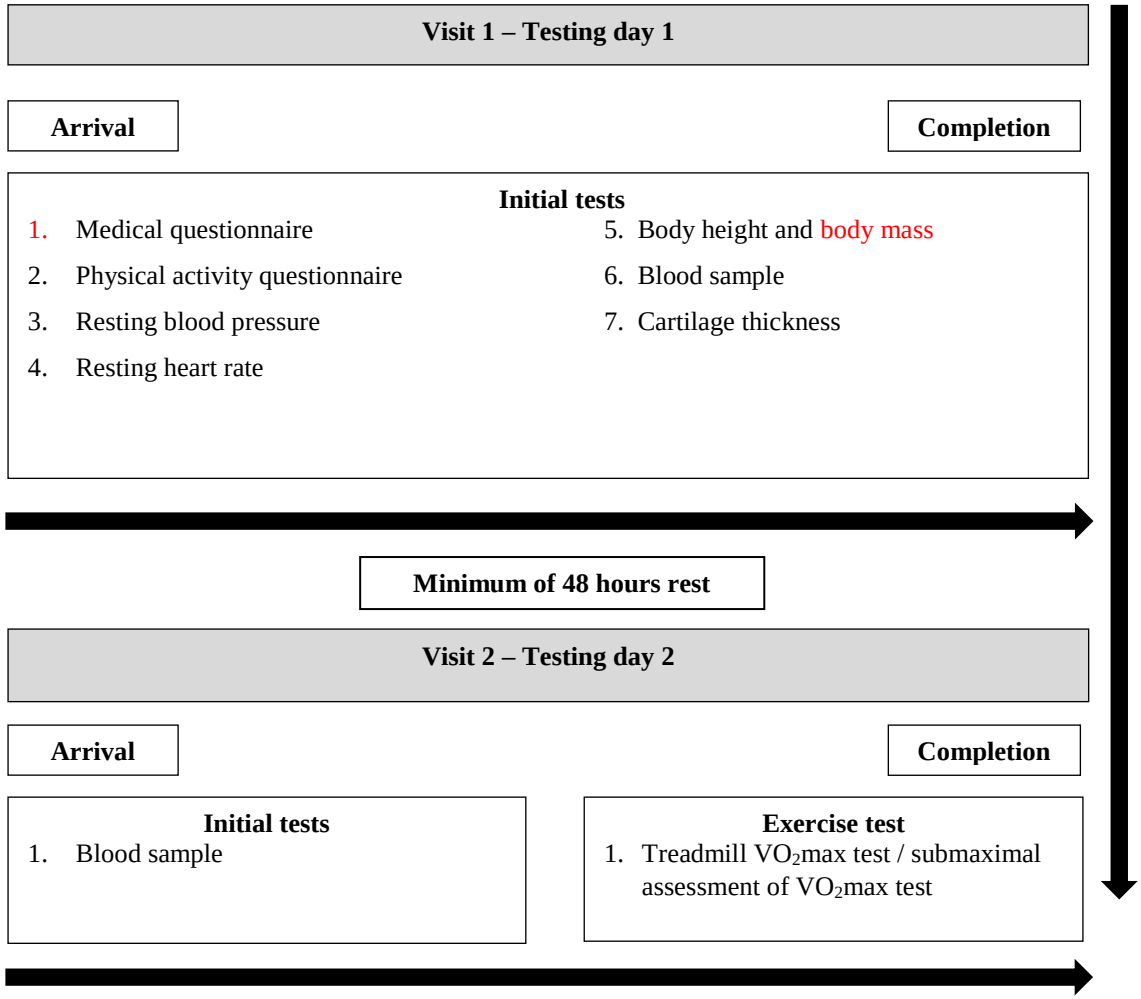


Figure 5-2 A schematic overview of the study protocol

Visit 1

On arrival to the department participants were seated and several questionnaires were completed. The initial questionnaire included questions relating to health and medical conditions, lifestyle, previous knee injuries and knee pain, as well as general physical activity and training metrics (Appendix D). Two specific physical activity questionnaires (7-day long IPAQ and Measurement of a Person's Habitual Physical Activity questionnaire) were also administered (Appendix A and B, respectively). Anthropometric measures, including, height and weight were also assessed. Following a period of 30 minutes of seated rest, one 6 ml blood sample was obtained via venepuncture to assess baseline serum COMP, HA, and

lubricin concentration (see Chapter 3). Ultrasonography was also used to obtain a resting measurement of femoral cartilage thickness (see Chapter 3).

Visit 2

On arrival to the laboratory participants were required to rest in a seated position for 30 minutes prior to providing a second resting blood sample. Participants subsequently completed either a submaximal or maximal incremental exercise test using a cycle ergometer to determine maximum oxygen uptake. The use of a submaximal or maximal exercise test was dependent on the American College of Sports Medicine (ACSM) risk stratification process (ACSM, 2010) (Appendix E).

5.3.3 Outcome measures

Blood collection and analysis

The two baseline samples (one from each visit) were used to establish a robust baseline value. Blood samples were collected on both visits following 30 minutes seated rest and at the same time of day to avoid the potential influence of circadian rhythms. Commercially available sandwich ELISAs were used to measure serum COMP, HA and lubricin concentrations as outlined previously. All assays followed the manufacturer's specifications and were performed within the institution (see Chapter 3).

Maximum Oxygen Uptake (VO_2 max)

To determine VO_2max, participants performed an incremental exercise test using the cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). Following an appropriate 4-minute warm-up, power output was increased using a ramp protocol at a pre-determined rate of 15, 20 or 25 Watts / min (which was dependent on the training status of each participant). The test continued until 85% of the age-predicted maximal heart rate was achieved for participants completing the submaximal test, or volitional exhaustion for those completing the maximal test. Throughout the cycling protocol participants were required to remain in a seated position and to maintain a cadence between 60 - 100 revolutions per min (rpm).

Physical activity questionnaires

To assess current levels of physical activity the IPAQ last 7-day long version was used (Craig et al. 2003). A modified version of the Measurement of a Person's Habitual Physical Activity questionnaire (Baecke et al. 1982), as used and validated by Pols et al. (1995), was also used

to assess physical activity over the last 12 months. This also included a work-related physical activity domain (work index) that was used an indicator of occupational stress. There is currently no 'gold standard' physical activity questionnaire to assess past physical activity, and thus, this study utilised these well-validated questionnaires that suited the population and cover both current and past physical activity levels. Further detail regarding the IPAQ 7-day long questionnaire and the Measurement of a Person's Habitual Physical Activity questionnaire are outlined in Chapter 3.

Acute knee injuries and previous knee pain

Individuals who reported a knee injury or suffered from chronic knee pain within the last 5 years were not included in the present study (see Chapter 3). Healthy participants who had sustained a knee injury prior to the last 5 years were required to detail this injury within the medical questionnaire during the first experimental visit (Appendix D). Participants were requested to report a knee injury if they had visited a medical professional and had needed to stop physical activity for a period of 2 weeks or more. Injuries that did not meet these criteria were considered 'minor' and were not reported. Participants who had not sustained a specific injury, but reported previous knee pain for a period of 2 weeks or more were also reported to detail this within Appendix D.

5.3.4 Sample Size Calculations

Sample size calculations were performed using PASS software (NCSS LLC, Kaysville, Utah) and with serum lubricin as the primary outcome variable. A sample size of 594 achieves 80% power to detect a change in a change in slope from 0.00 under the null hypothesis to 0.10 under the alternative hypothesis when the SD of the X (age) is 25.00, the SD of Y is 21.85 (serum lubricin), and the two-sided significance level is 0.05. To detect a change of in slope from 0.00 to 0.30 a more feasible sample size of 61 was specified. Given that there remains limited literature to provide a clear indication of the expected relationship between the serum biomarkers and age, this study was termed 'exploratory' and targeted a recruitment total of a minimum of 60 participants.

5.3.5 Statistical analysis

Right femoral cartilage thickness was used for all data analysis as side to side differences in cartilage thickness are understood to be minimal (see Chapter 4). Pearson correlations

(parametric data) and Spearman's rank correlations (non-parametric data) were initially performed to examine the relationships between all baseline continuous variables. To assess the first hypothesis simple regression was used to explore the relationship between ageing and the dependent variables. Hierarchical multiple regression models were subsequently used to explore the relationship between each dependent variable and age, after adjusting for body weight, injury, physical activity over last 7 days and physical activity over last 12 months. As appropriate follow-up moderation analyses were conducted using the SPSS PROCESS syntax developed by Hayes, (2012). An interaction was deemed present if a significant interaction between the independent variables was observed. Bootstrapped analyses, based on a 1000 samples, were used if model assumptions were violated and robust regression was required. In addition to test the second hypothesis, simple regression was used to explore the relationship between measures of physical activity (7-day IPAQ scores, 12-month physical activity score, and VO_2max) and the dependent variables. Exploratory analyses were also completed to determine whether differences existed between participants with cartilage thickness less than the 25^{th} percentile, hereafter referred to as 'thin' cartilage, and participants with cartilage thickness greater than the 25^{th} percentile, referred to as 'thick' cartilage. Independent t-tests were used to determine between groups differences (thin vs thick cartilage).

5.4 Results

In total 81 participants completed the study, forming an excellent cross-sectional sample of healthy male individuals. The purpose of this cross-sectional sample was to provide a large sample of healthy males with a range of ages and physical activity levels. Physical and baseline characteristics are shown in Table 5-1.

Table 5-1 Physical characteristics of participants

	Men (n = 81)	
Variable	**Mean (SD)**	**Range**
Age (years)	43 (16)	18 - 70
Height (m)	1.79 (0.07)	1.64 - 1.95
Body mass (kg)	79.4 (8.6)	63.3-106.2
BMI (kg/m^2)	25.2 (2.4)	21.0-30.6
VO_2max (mL·kg·min^{-1})	44.6 (12.0)	24-67
7 day IPAQ score (MET-min/week)	4604 (4882)	400-24316
12-month physical activity index	8.2 (1.7)	5.0-12.6
Serum COMP (log COMP)	6.6 (0.2)	6.1 - 7.2
Serum Lubricin (ng/ml)	112.8 (43.4)	24.3 – 201.8
Serum HA (ng/ml)	20.6 (18.7)	3.1 – 73.3
Knee cartilage thickness (mm)		
Right intercondyle notch	2.18 (0.42)	1.28 – 3.38
Right medial condyle	1.98 (0.37)	1.27 – 2.93
Right lateral condyle	1.93 (0.30)	1.41 – 2.73

5.4.1 Ageing

Age was positively associated with serum COMP (β = +0.005/year, P < 0.01) and negatively associated with cartilage thickness at the lateral condyle (β = -0.007/year, P < 0.01) (Figure 5.3). Following the adjustment for weight, previous injury and physical activity level, only the negative association with lateral condyle thickness remained (β = -0.007/year, P < 0.01). Hierarchical analysis revealed that age accounts for 9.3% of the variability in lateral cartilage thickness (R^2 change = 0.093, P = 0.01), over and above the variability accounted for by weight, previous injury, 7-day physical activity level, 12-month physical activity level. However, age was not associated with either serum HA, serum lubricin, or cartilage thickness at the intercondyle notch or medial condyle. Results demonstrated that neither recent 7-day activity, activity over the last 12 months, or VO_2max moderated the age-associated decrease in lateral condyle cartilage thickness (all, P > 0.05). A graphical illustration of the interaction plot for the moderated regression analyses are provided, see Figure 5.4.

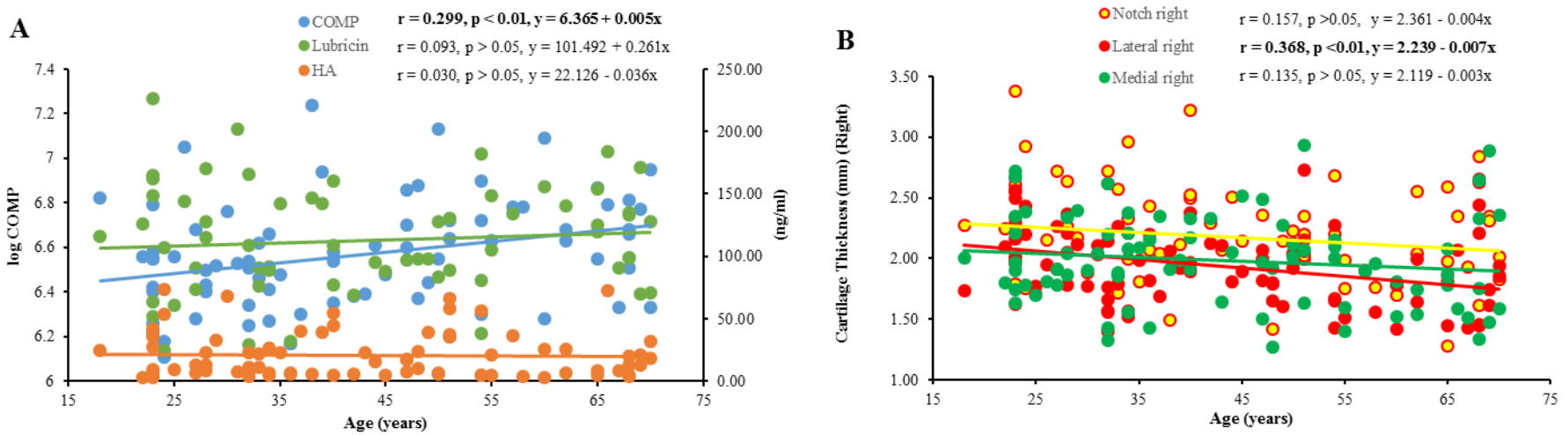


Figure 5-3 The association between age and dependent variables; A) serum biomarkers, B) right femoral cartilage thickness. Significant relationships are highlighted in bold

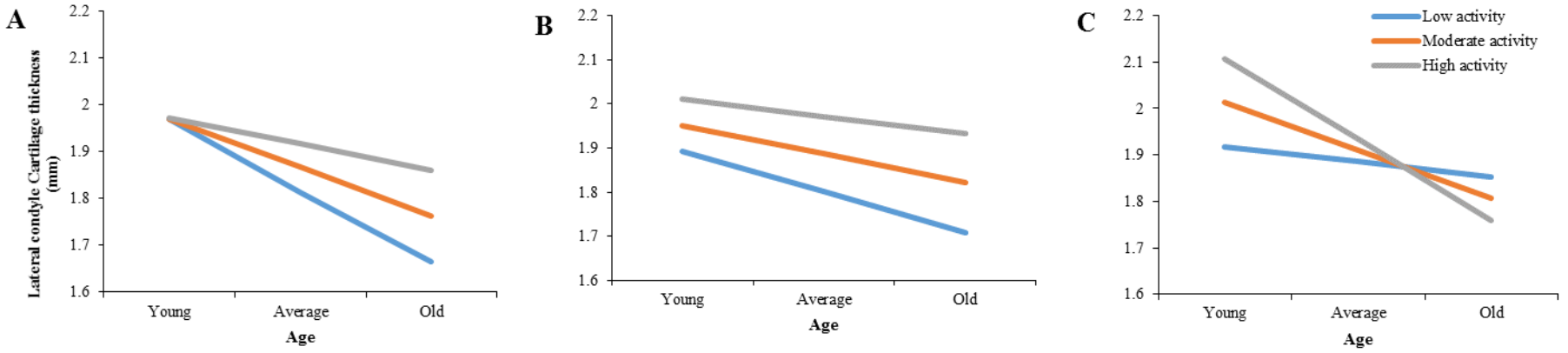


Figure 5-4 An interaction plot to demonstrate the moderating effects of physical activity on the association between age and lateral condyle cartilage thickness; moderating effects of A) 7 day IPAQ score; B) 12-month physical activity score; and C) VO_2max

5.4.2 Physical activity level

Simple regression analysis demonstrated a significant positive relationship between serum COMP values and the amount of physical activity over the previous 7 days ($P < 0.05$); however, after adjusting for age, weight and previous injury the significant association was no longer evident. Similarly, alternative measures of activity, including, physical activity over the last 12 months, and VO_2max, were not significantly associated with serum COMP (Figure 5-5B) Likewise, there was no significant association between serum HA or serum lubricin and any measure of activity (Figure 5-5A). Cartilage thickness at the lateral condyle was positively associated with both physical activity over the last 12 months and VO_2max (both, $P < 0.05$). Furthermore, there was positive association between cartilage thickness at the medial condyle and last 7-day physical activity and activity over the last 12 months ($P = 0.06$ and $P = 0.07$, respectively). There were no significant association between cartilage thickness at the intercondyle notch and any measure of physical activity. Importantly, following the adjustment of age, weight, and previous injury only physical activity over the last 12 months demonstrated a potential positive relationship with lateral cartilage thickness ($P = 0.05$).

5.4.3 Weight, BMI and height

Simple regression analysis revealed a significant negative association between weight and lateral condyle cartilage thickness ($P < 0.05$). Similarly, there was a significant negative association between BMI and lateral condyle thickness ($P < 0.01$). Following the adjustment of potential cofounders, including age, injury, and physical activity over the last 12 months, weight did not significantly predict lateral cartilage thickness; in contrast, BMI significantly accounted for 8.9% of the variance in lateral condyle cartilage thickness ($P < 0.05$). No other significant associations were demonstrated between weight, BMI, or height and any of the other dependent variables.

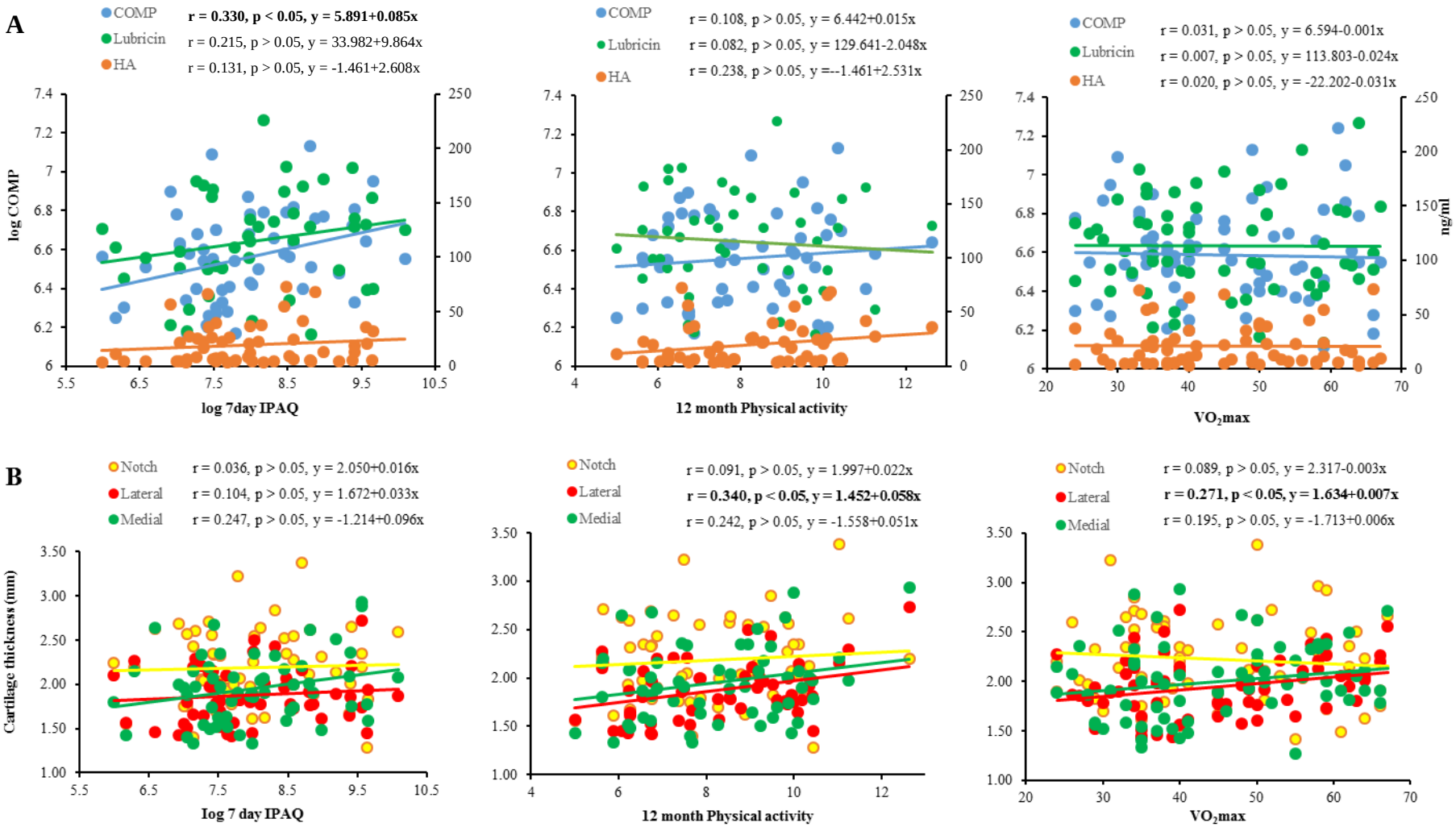


Figure 5-5 A) The association between indices of physical activity and serum biomarkers; B) The association of physical activity indices and right femoral cartilage thickness at intercondyle notch, lateral condyle and medial condyle. Significant relationships are highlighted in bold

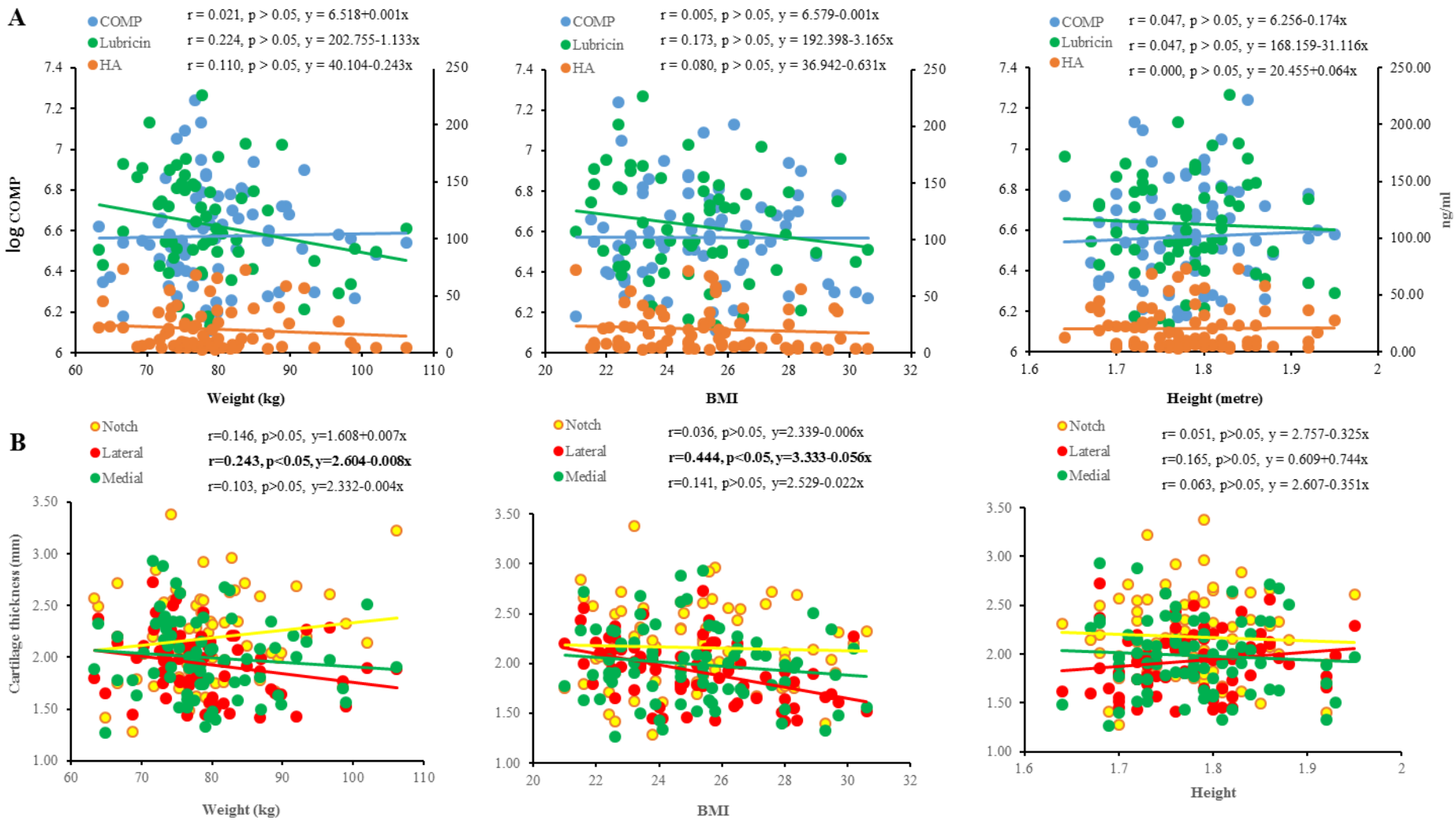


Figure 5-6 A) The association of weight, BMI, and height and serum biomarkers; B) The association of weight, BMI, and height and right femoral cartilage thickness at intercondyle notch, lateral condyle and medial condyle. Significant relationships are highlighted in bold

5.4.4 ‘Thin’ and ‘thick’ cartilage

To explore potential differences in individuals with ‘thin’ and ‘thick’ femoral cartilage thickness, the present cross-sectional sample was split into two groups based on the cartilage thickness of the femoral intercondyle notch. Based on the quartile split, individuals with a femoral intercondyle notch thickness of less than or equal to 1.90 mm were considered ‘thin’ and individuals greater than or equal to 2.52 were considered ‘thick’. This produced a sample of twenty individuals per group. There were no significant differences between the thin and thick groups in physical characteristics, measures of physical activity and fitness, and serum biomarkers (Table 5-2; all $P > 0.05$). However, the ‘thin’ cartilage thickness group did consist of a greater number of individuals with both knee pain, as well as a family history of OA, compared to the ‘thick’ cartilage thickness group.

Table 5-2 Presents data for 'thin' vs 'thick' femoral intercondyle cartilage thickness groups

Variable	Thin (n = 20)		Thick (n = 20)	
	Mean	SD	Mean	SD
Age (years)	44	16	40	18
Height (metres)	1.78	0.07	1.78	0.90
Body mass (kg)	77.6	8.0	80.3	10.2
BMI	24.8	2.4	24.8	2.1
Joint load (height*weight)	138.0	17.5	143.1	20.3
VO_2max ($mL \cdot kg \cdot min^{-1}$)	47	12	42	11
7 day IPAQ score (MET-min/week)	5080	5344	4968	5924
12-month physical activity index	8.0	1.6	8.4	1.7
Work index	2.3	0.6	2.5	0.9
No. in high impact/risk work	1/20 (5%)		2/20 (10%)	
No. with acute injury	5/20		4/20	
No. with knee pain	6/20		3/20	
Family history of OA	4/15 (25%)		0/16 (0%)	
COMP (log)	6.60	0.26	6.55	0.18
Lubricin (ng/ml)	126.3	45.7	102.5	47.9
HA (ng/ml)	17.4	20.3	25.6	28.2

5.5 Discussion

This cross-sectional study demonstrates that ageing in healthy male individuals is not associated with a significant change in serum COMP, HA or lubricin. Furthermore, age is not associated with a decrease in femoral cartilage thickness at either the intercondyle notch or medial condyle. These results indicate that, in a cohort of healthy individuals, ageing has a no effect on cartilage turnover or joint lubrication. However, age was associated with a small but significant reduction in lateral condyle cartilage thickness. It is possible that the reduction in lateral condyle cartilage thickness is related to age-associated biomechanical differences in loading of the femoral cartilage compartments. Interestingly, although a greater level of physical activity level over the last 12 months was associated with increased cartilage thickness, physical activity did not appear to moderate the age-related decline in cartilage thickness.

Ageing, a known risk factor of OA, has previously been associated with an increase in both serum COMP (Clark et al. 1999) and serum HA (Elliott et al. 2005). These changes are indicative of early knee degeneration and have previously been associated with knee OA (Elliott et al. 2005; Verma and Dalal, 2013). In the present study, age could not explain a significant variation in either serum COMP, HA or lubricin, following adjustments for body weight, previous injury, and physical activity level. These biochemical indices suggest that healthy males are not subject to increased cartilage and synovial tissue turnover, or a reduction in the joint lubrication compared to their younger contemporaries. However, in the present study ageing was found to be negatively associated with lateral femoral condyle cartilage thickness. This supports several previous research studies that have also indicated that ageing is associated with a reduction in knee cartilage morphology, including femoral cartilage thickness (Hudelmaier et al. 2001), as well as lateral condyle thickness assessed by ultrasonography (Özçakar et al. 2014). It is possible that the negative association between age and lateral femoral condyle cartilage thickness is linked to early degenerative change (Karvonen et al. 1994). Possible reasons for age-associated decrease in lateral femoral condyle cartilage thickness are less clear. Differences in the age-related change across the various locations of the femoral cartilage suggests that there is a potential modification in the distribution of load across the knee joint with age. One possibility is that valgus alignment and a concomitant reduction in knee stability could increase lateral loading of the femoral cartilage (Andriacchi, 2013). In this case, correcting the joint alignment in combination with

muscle strengthening across the knee joint may be important for cartilage health, particularly in older individuals. Moreover, several studies have provided evidence that joint loading is important for cartilage morphology (Foley et al. 2007; Racunica et al. 2007; Vanwanseele et al. 2002). Therefore, the results of the present study may reflect a reduction in the cyclic loading of the lateral femoral condyle, possibly because of an age-related reduction in physical activity and increase in BMI. These results may also relate to this sample, which were male, predominately of healthy weight, and free from injury. Gender, obesity and serious knee injury are major predictors of knee OA and may moderate the relationship between age and both serum biomarkers and cartilage thickness.

There remains much debate regarding physical activity and the risk of knee OA. Several studies have indicated that individuals of all ages can tolerate moderate amounts of exercise without significant degenerative change (Esser and Bailey, 2011; Urquhart et al. 2011), and indeed, a number of studies indicate that moderate loading may actually have a positive effect on joint function (Foley et al. 2007; Racunica et al. 2007). It was hypothesised that physical activity would be protective and would help mediate any negative age-related changes. However, in the present study physical activity did not appear to moderate the age-associated change in lateral cartilage thickness; although, overall a limited age-associated change was observed among all dependent variables. Hence, to explore the effect of physical activity further exploratory analyses were completed. Results demonstrated a significant positive association between serum COMP and physical activity over the last 7 days. However, following adjustment for age, weight and injury there was no association. Furthermore, no associations were determined between indices of physical activity (i.e. physical activity over the last 12 months, and current VO_2max) and serum COMP, HA, or lubricin. Therefore, in this healthy sample, physical activity does not appear to significantly alter, either positively or negatively, baseline cartilage turnover, synovial tissue turnover, or joint lubrication. Although previous research has demonstrated that serum biomarkers, COMP and HA are sensitive to acute physiological loading (Mündermann et al. 2005; Pruksakorn et al. 2013), there remains limited research exploring physical activity history and these baseline biochemical indices and further work is required.

Cartilage thickness at the femoral lateral condyle correlated positively with activity over the last 12 months and VO_2max. Similarly, there was also a trend towards a positive relationship between cartilage thickness at the medial condyle and both recent physical activity and

activity over the last 12 months. However, following the adjustment of age, weight, and previous injury, only activity over the last 12 months remained positively associated with lateral cartilage thickness (P = 0.05). Several previous studies have failed to demonstrate a relationship between individuals with a history of high levels of knee joint loading and an increase in cartilage morphology (Gratzke et al. 2007). In contrast, regular vigorous weight-bearing physical activity has previously been associated with both increased tibial cartilage volume and inversely associated with cartilage defects (Racunica et al. 2007). Similarly, greater muscle strength and endurance fitness has previously been associated with a reduction in the rate of cartilage loss (Foley et al.
2007), while strenuous exercise has been implicated in the protection against lateral knee cartilage defects (Foley et al. 2007). While studies indicate that physical activity may be protective, the present study failed to provide any strong evidence that physical activity either moderates the age-related decline in lateral cartilage thickness or has a particularly strong effect on cartilage thickness and serum markers. This suggests that while physical activity appears to be well tolerated, any potential benefits are modest in healthy individuals, and may be limited to certain cartilage locations.

In addition to physical activity, knee joint loading is influenced by a person's physical characteristics. Body weight, body height and BMI all play a role in knee joint loading, and consequently may impact upon knee articular cartilage morphology. Of the exploratory analyses conducted, cartilage thickness at the lateral condyle was negatively associated with both body weight and BMI. However, only the negative association with BMI remained once the association was adjusted for potential cofounders. The negative association between BMI and lateral femoral condyle thickness is consistent with Chapter 4 and supports the hypothesis that increases in joint loads associated with a higher BMI may result in a greater risk of degenerative change. However, the relationship between BMI and cartilage thickness has been inconsistent (Ding et al. 2005). Furthermore, others have reported a positive relationship between BMI and femoral cartilage thickness in young individuals (Blazek et al. 2014). Differences between the relationship of either body weight or BMI and cartilage thickness is likely to may be dependent on factors such as age and whether the joint is comprised (e.g. conditions just as joint malalignment or OA). Neither body weight, body height nor BMI were associated with any of the serum biomarkers in the current study. Therefore, among healthy men, body weight and BMI have no effect on markers associated with cartilage metabolism, lubrication and has little effect on cartilage thickness. Further

work is required to identify whether age and joint health alters the relationship between anthropometric variables and cartilage markers.

Previous analyses suggest that neither age, physical activity level nor anthropometric measures are strongly related with differences in cartilage thickness, particularly when the intercondyle notch and medial condyle are considered. While it is attractive to think that 'thicker' femoral cartilage is favourable compared to 'thinner' cartilage, it remains unknown whether cartilage thickness influences long-term knee health. Therefore, exploratory analyses were also completed to compare several variables in a subset of individuals with 'thin' and 'thick' intercondyle notch cartilage. The results revealed that 'thinner' femoral cartilage thickness was not related to a difference in age, weight, BMI, joint load, physical activity level, or serum biomarker concentrations, but was associated with a greater number of individuals reporting knee pain, a family history of OA, and previous knee injury. However, further analysis of participants who had previously sustained a knee injury did not reveal any difference in serum biomarker profile or a reduced femoral cartilage thickness compared to participants who had not sustained a previous injury (see Appendix F). Knee pain and stiffness has previously been associated with reduced cartilage thickness assessed by supra-patellar ultrasonography (Abraham et al. 2014). While it is rational to associate reduced cartilage thickness with knee pain, findings are inconsistent. For example, radiographic knee OA is often considered an imprecise guide as to the likelihood and severity of knee pain (Bedson and Croft, 2008). This imprecision is most likely since osteoarthritic knee pain is a multifaceted, and can involve mechanical, structural, inflammatory, bone-related, neurological and psychological factors (Hunter et al. 2009).

The serum biomarkers (COMP, HA and Lubricin) and femoral cartilage thicknesses in the present study demonstrated considerable variability across individuals. As expected the baseline serum COMP concentrations in the present study were considerably lower compared to previous work in RA patients from within the same research group (767 vs 1347 ng/ml) (Law et al. 2015) and in patients with OA (767 vs 890-4100 ng/ml) (Jordan et al. 2003; Senolt et al. 2005). Similarly, baseline serum HA concentrations in the present study were also lower than previously reported in RA (21 vs 402 ng/ml) (Engström-Laurent and Hällgren, 1987) and OA (21 vs 258) (Criscione et al. 2005). However, both serum COMP and HA concentrations are comparable to serum concentrations previously reported in healthy individuals (Denning et al. 2015; Wakitani et al. 2010). Importantly, this is the first

study to report serum lubricin values in a sample of healthy males, thus providing the first normative data for a cross-section of healthy individuals. Although serum biomarkers, including serum COMP and HA, have previously been associated with clinical measures and symptoms (Clark et al. 1999; Inoue et al. 2011), in the present study there was no correlation between serum biomarkers and femoral cartilage thickness assessed by ultrasonography. Previously, serum COMP concentrations have been correlated with the radiographic severity and MRI score in patients with knee OA (Clark et al. 1999; Sharif et al. 1995b; El-Arman et al. 2010). It is possible that ultrasonography does not offer the precision required to detect minor changes to cartilage in healthy individuals. Studies investigating serum biomarkers should attempt to develop a greater understanding of the relationship between biochemical indices and both structural changes and clinical outcomes. Future cross-sectional studies exploring cartilage thickness and serum biomarkers would also benefit from considering dietary composition of individuals. Although several supplements including, glucosamine, chondroitin and fish oils were controlled for within the present study. A limitation of the present research was the lack of control within the study design for differences in food sources such high in fish oils and vitamin D that may influence the outcome measures in the present study.

Moreover, there remains a paucity of normative data surrounding both the cartilage thickness and serum biomarker response to acute loading. The response of biomarkers to acute loading provides provide an additional measure of joint health and may also be predictive of structural change. In addition, the knowledge of what constitutes a ‘normal’ response is also important to identify potential ‘abnormal’ or ‘unhealthy’ responses. Thus, future studies should expand this normative data and identify the exercise-induced response of both serum biomarkers and femoral cartilage thickness to acute loading in healthy individuals. In addition, although the current study indicates that higher levels of physical activity does not adversely affect the joint, further research is required to investigate how different modalities of exercise (e.g. weight bearing vs non-weight bearing; high repetition loading vs load repetition loading) affect serum biomarkers and femoral cartilage thickness in both healthy males and females. These questions will be explored in the following chapters.

5.6 Conclusion

The present study, together with Chapter 4, has provided a wealth of normative data relating to femoral cartilage thickness and serum biomarkers in healthy individuals across a wide ride of ages and physical activity levels, which to date, has not been previously been reported. In healthy males between the age of 18-70 years, ageing was not associated with the serum biomarker profile or femoral cartilage thickness at either the medial condyle or the intercondyle notch. The only significant association with age was limited to a negative association with cartilage thickness at the lateral condyle. Overall, the results of the current study provide evidence to suggest that healthy ageing is not associated with any degenerative change and further supports the normative data from Chapter 4. Furthermore, in contrast to the second hypothesis, physical activity did not moderate the age-related negative association with lateral cartilage thickness. Moreover, the association between 12-month physical activity levels and lateral condyle cartilage thickness suggests that joint loading associated with higher level of physical activity may increase or maintain cartilage morphology in this healthy cohort. These results indicate that physical activity is both well tolerated and potentially beneficial for lateral condyle cartilage thickness. In relation to the effect of anthropometrics measures, only BMI was consistently associated with thinner cartilage in both the current study and in Chapter 4. This suggests that the lateral condyle may be the location most at risk of change among individuals with higher BMI. However, overall, both the current study and Chapter 4 demonstrated that anthropometrics measures are not often associated with either femoral cartilage thickness or serum biomarkers in healthy individuals.

Study contributors:

Harry Roberts, A/Prof Jeanette Thom and Dr Jonathan Moore conceived and designed study; Harry Roberts and Lewis Angell collected raw data; Harry Roberts and Jason Edwards completed the preparation and analyses of blood samples; Harry Roberts completed all data analyses and prepared chapter.

6 CHAPTER 6. The effect of vigorous running and cycling on novel markers of knee joint function

6.1 Abstract

PURPOSE: The aim was to investigate lubricin, COMP and femoral cartilage deformation in response to different biomechanical loading of the knee joint (running *versus* cycling).

METHODS: Serum lubricin and COMP concentrations (ELISA), and femoral cartilage thickness **(**supra-patellar transverse ultrasonography) were determined in 11 male runners (age: 40 ± 6 years; body mass: 76 ± 8 kg) and 11 male cyclists (35 ± 12 years; 75 ± 5 kg) at baseline, immediately after, and 30 minutes after vigorous exercise (time trial: 10 km run or 25 km cycle).

RESULTS: At baseline, lubricin (runners: 104.9 ± 29.5 ng/ml; cyclists: 114.1 ± 41.8 ng/ml) and COMP (runners: 804.1 ± 259.3 ng/ml; cyclists: 693.9 ± 189.0 ng/ml) did not significantly differ, however, vigorous exercise was accompanied by an increase in lubricin (cyclists: 45.6%; $P < 0.05$; runners: 55.5%; $P < 0.05$) and COMP (cyclists: 32.1%; $P < 0.05$; runners: 14.2%; $P = 0.14$) that returned towards baseline following 30 minutes of rest ($P < 0.05$). No between-group differences were observed for baseline cartilage thickness at the intercondyle notch, medial condyle and lateral condyle, and vigorous exercise did not result in significant change for either group.

CONCLUSIONS: In the absence of ultrasonographic knee cartilage deformation, the response of serum lubricin and COMP following acute vigorous exercise indicates an increase in joint lubrication and cartilage metabolism, respectively, which appears largely independent of exercise modality.

6.2 Introduction

Regular exercise has been shown to preserve knee cartilage volume and thickness (Mosher et al. 2010; Racunica et al. 2007) reduce cartilage defects (Racunica et al. 2007), increase proteoglycan content (Van Ginckel et al. 2010) as well as reduce knee disability and pain (O'Reilly et al. 1999). While regular moderate joint loading may be chondroprotective and promote healthy knee joint function (Urquhart et al. 2011), high levels of joint loading may result in negative adaptations (Driban et al. 2015). Weight bearing activities such as running are associated with much larger lower-body peak forces (D'Lima et al. 2008) and may be subject to an increased risk of OA compared to non-weight bearing activities such as cycling (Franciozi et al. 2013; Vignon et al. 2006). To date, there remains a paucity of research regarding the effect of acute running and cycling exercise on overall joint function.

Although mechanical loading is required for tissue maintenance and metabolism, chronic abnormal loading, exaggerated by obesity, joint malalignment or high levels of physical activity, have been implicated as extrinsic risk factors for cartilage damage (Guilak, 2011). Articular cartilage is known to deform with exercise (deformational behaviour) before returning to pre-loading values with rest (Kessler et al. 2006). The magnitude of deformation differs between various types of exercise, is 'dose dependent', and may to some extent be related to mechanical differences associated with exercise (Eckstein et al. 2005). Deformational behaviour is also understood to provide unique information regarding the biochemical composition of the cartilage tissue (Eckstein et al. 2006) and has previously been correlated with an exercise-induced increase in COMP (Kersting et al. 2005).

Clinical measures of early knee joint adaptation are elusive. However, easily accessible serum biomarkers are understood to reflect the release of molecules or molecular fragments from the loaded joint and are increasingly acknowledged as a method to monitor early joint adaptation, degenerative change, and OA (Bauer et al. 2006). Specifically, this study will utilise serum COMP and serum lubricin (see Chapter 2) to investigate the response of the knee joint to acute loading through either running or cycling exercise. In addition to serum biomarkers, US of the knee joint was utilised to provide a valid and reliable measure of femoral cartilage thickness at three identifiable locations, the intercondyle notch, medial condyle and lateral condyle (Naredo et al. 2009), both before and after exercise.

The sonographic evaluation of cartilage thickness, together with biomarkers lubricin and COMP, offer an attractive method to explore the physical and biochemical tissue response following an acute bout of running and cycling. Therefore, the aims of this study was to explore the effects of a single session of fatiguing exercise on novel markers of cartilage turnover and joint lubrication, and cartilage thicknesses. Of additional interest was whether trained runners respond differently to trained cyclists following a fatiguing exercise protocol which was specific to their activity and suitably matched for intensity and duration.

Hypothesis 1: Acute fatiguing exercise would result in an exercise stimulated increase in serum concentration of COMP

Specific objective 1: To test the second hypothesis, a blood sample was obtained via venepuncture pre-exercise, immediately following and 30 minutes. Serum concentrations of COMP was determined using commercially available ELISAs

Hypothesis 2: Acute fatiguing exercise would result in an exercise stimulated change in serum concentration of lubricin

Specific objective 2: To test the second hypothesis, a blood sample was obtained via venepuncture pre-exercise, immediately following and 30 minutes. Serum concentrations of lubricin was determined using commercially available ELISAs

Hypothesis 3: Acute fatiguing exercise will result in a greater increase in exercise stimulated serum COMP in runners compared to cyclists.

Specific objective 3: To test the third hypothesis, a blood sample was obtained via venepuncture pre-exercise, immediately following and 30 minutes. The change in serum concentrations of COMP was compared between runners and suitably matched cyclists.

6.3 Methodology

6.3.1 Participants

A homogeneous group of healthy trained runners and cyclists were recruited for this study. To maintain a homogeneous sample, the inclusion criteria for entry to the study included being male, aged between 18-59 years, and a BMI of < 30 kg / m^2. Runners were required to have recently completed a 10-km time trial in under 45 minutes and/or regularly complete > 25 miles per week in training. In comparison, cyclists were required to have recently completed a 25-km time trial in under 45 minutes and/or regularly complete > 80 miles per

week in training and / or regularly cycle > 4 hours per week. Cyclists were also required to have a limited history of weight bearing activity and have not completed regular weight bearing sports training or running within the last 6 months. Exclusion criteria for both groups are detailed in Chapter 3. A control group was not included within this study design due to the difficulties of finding an equally well matched group of trained individuals who did not engage in weight bearing or non-weight bearing activities. Details of the recruitment process through to study completion are outlined in Figure 6-1.

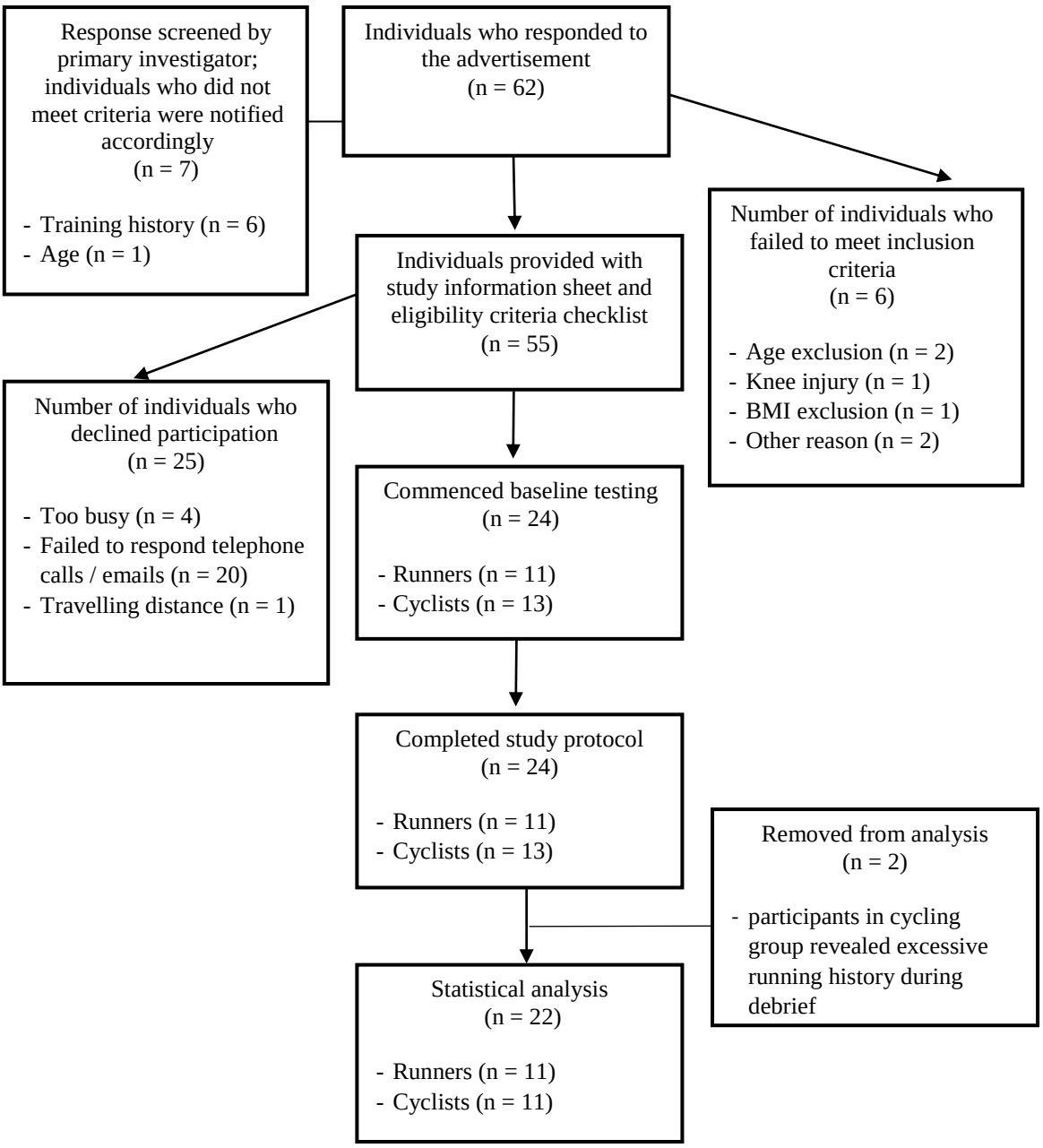


Figure 6-1 Participant flow diagram detailing participant recruitment, baseline testing and protocol completion

6.3.2 Experimental protocol

In this two group designed study, participants were required to visit the physiology laboratories in SSHES, Bangor University on two separate occasions, with a minimum of 48 hours between visits and a maximum of 2 weeks, as outlined below in Figure 6-2.

Visit 1

During the first visit, height, body mass and a resting venous blood sample were obtained as specified in Chapter 3. Participants subsequently completing an incremental exercise protocol to determine maximum oxygen uptake (VO_2max).

Visit 2

During the second visit participants provided an additional resting venous blood sample and underwent US scanning of the femoral cartilage (to assess cartilage thickness) of the right leg, before, immediately post, and 30 minutes post the vigorous time trial protocol. Participants were required to limit their training / racing prior to each visit and were unable to take part in the study if they had taken part in a marathon or ultra-distance event within 7 days of a testing day. Training within the 48 hours prior to testing was limited to 1 hour of moderate intensity training and participants had to refrain from training in the 24 hours prior to testing. Participants also provided detailed information regarding their typical training (frequency, intensity, duration and type) and provided information regarding their training history (number of years of training).

Time-trial exercise protocol

The vigorous exercise protocol was specific to the participant's activity: trained runners completed a 10-km running time trial using the treadmill ergometer and trained cyclists completed a 25-km cycling time trial using a cycle ergometer. Runners self-regulated their speed using the treadmill monitor, while the gradient remained standardised at 1% to account for the lack of wind resistance. Similarly, cyclists also adopted a self-selected workload and cadence. Although participants self-regulated their workload, travelling speed and exercise-time were not provided. Importantly, participants were instructed to complete the time-trial protocol as intensely as possible to mimic actual competition. Continuous verbal encouragement was provided throughout. Both heart rate and RPE measurements were taken

at several time points throughout the trial to ensure the protocol replicated competitive conditions

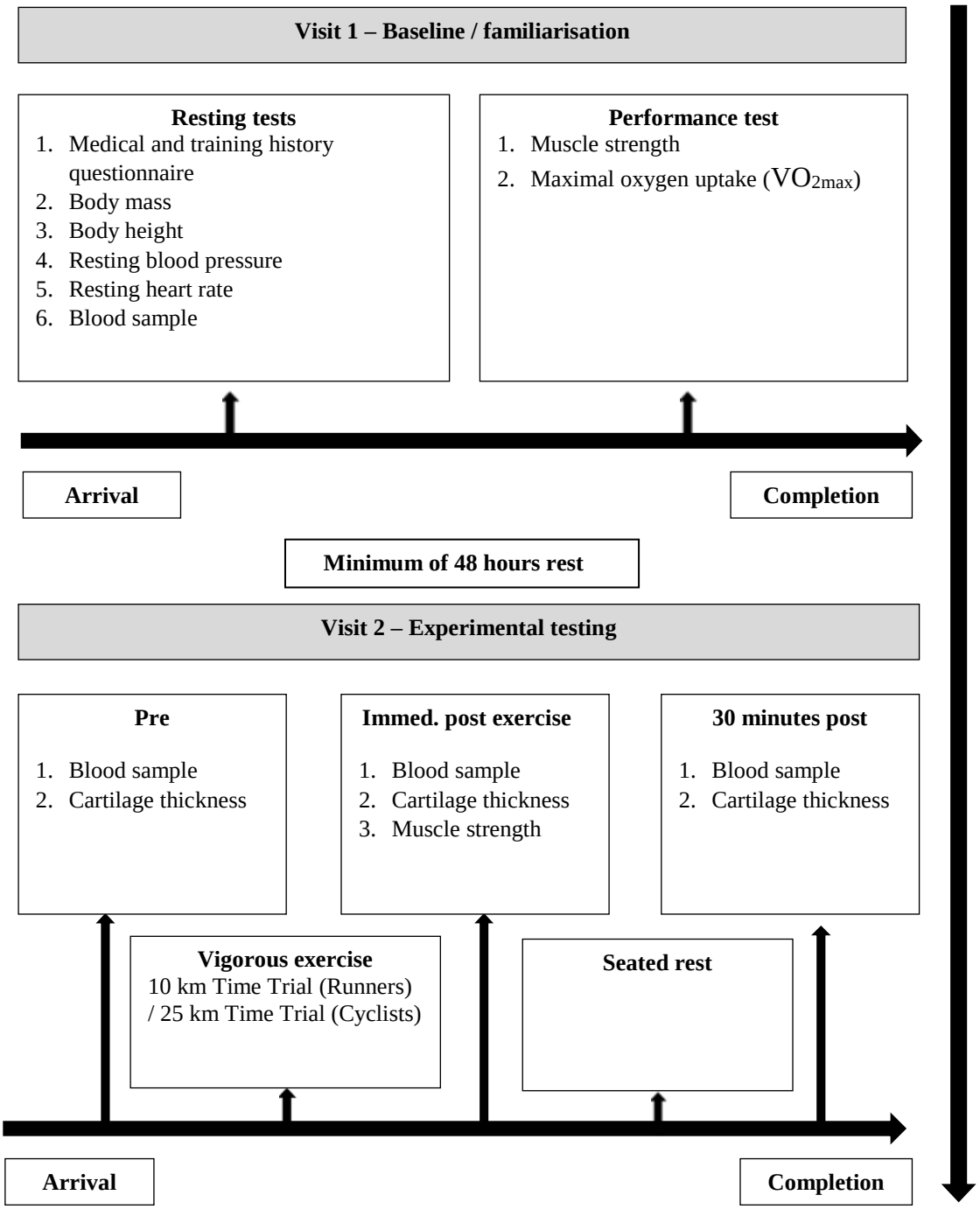


Figure 6-2 A schematic overview of the study protocol

6.3.3 Outcome measures

Blood collection and analysis

In total, four blood samples were obtained across the two visits. The two baseline samples (one from each visit) were used to establish a robust baseline value. Blood samples were collected on both visits following 15-minutes of seated rest and at the same time of day to avoid the potential influence of circadian rhythms. During visit two, blood samples were also obtained immediately post exercise and 30-minutes post exercise cessation. Commercially available sandwich ELISAs were used to measure serum COMP and lubricin concentrations as outlined previously in Chapter 3.

Maximum oxygen uptake (VO_2max)

To determine VO_2max, participants performed an incremental exercise test that was specific to their activity group using the treadmill (HPcosmos Mercury 4 Med, Nussdorf-Traunstein, Germany) or cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). For runners using the treadmill, an appropriate warm-up was followed by an incremental exercise protocol, which started at an intensity appropriate to the participants' training status. Every 3 minutes, the intensity was increased until cessation of the test. For runners completing the treadmill ergometer test the speed was increased by 1 km / hour until participants reached 16 km / hour, with any further increases in intensity achieved through a 3% increase in gradient every 3 minutes. For cyclists using the cycle ergometer, following an appropriate 4-minute warm-up, power output was increased using a ramp protocol at a pre-determined rate of 25 Watts / min. Throughout the cycling protocol participants were required to remain in a seated position and to maintain a cadence between 60 - 100 rpm. For both protocols, heart rate was monitored continuously using a heart rate monitor (Polar Electro, Kempele, Finland) and the RPE was measured using the Borg scale during the last 15 seconds of each stage (Borg, 1982) (see Chapter 3). Both testing protocols continued until volitional exhaustion.

Sonographic assessment of cartilage thickness, deformation and recovery

Methodological details regarding the image capture and analysis are specified in Chapter 3. Cartilage thickness deformation and recovery was calculated as the difference in cartilage thickness between the measure obtained pre-exercise and immediately-post the acute exercise protocol and the measure taken immediately-post and post 30 minutes of seated rest, respectively.

6.3.4 Sample size calculation

Sample size calculation were conducted prior to commencing testing using G*Power 3.1.3 (Heinrich-Heine-University) software. Assuming an alpha value of 0.05 and 80% power, a total sample size of 24 participants was calculated to detect a moderate effect size (0.50).

6.3.5 Statistical analysis

Statistical analyses were performed utilising statistical analysis software (SPSS for Windows version 20.0 [SPSS, Chicago, IL, USA]). A two-factor (treatment x time) repeated-measures analysis of variance was used to assess the within groups difference (baseline vs. immediately post-exercise vs 30-minute post-exercise), between group difference (runners vs. cyclists) and time by treatment interactions (group differences over time) for serum lubricin, serum COMP, and femoral cartilage thickness. A single baseline value (average of visit 1 and visit 2) was used for within group analysis of serum lubricin and COMP to determine a robust baseline value and the coefficient of variation was calculated. Significant interactions and/or main effects were analysed post-hoc using Bonferroni-corrected t-tests where appropriate. Independent sample t-tests were used to assess differences between runners and cyclists. Pearson correlations (parametric data) and Spearman's rank correlations (non-parametric data) were performed to examine the relationships between all baseline continuous variables. As appropriate, sensitivity analysis was completed to determine the effect of outliers and to strengthen the conclusions drawn from the analyses. Effect sizes were calculated using the Cohen d equation; 0.2, 0.5 and 0.8 were considered as small, moderate and large effect size, respectively. Normality of data was explored by visual inspection of Q-Q plots and through analysis of the model's residuals. All figures and tables are presented as mean ± SD, with statistical significance set as ($P < 0.05$).

6.4 Results

Of the twenty-four participants who completed the study, two cyclists revealed a history of weight-bearing sporting activity and were consequently removed from study. Therefore, in total, twenty-two participants (running group: n = 11; cycling group: n = 11) were included within the analyses. Both groups were comparable for the number of training years, average number of days and average number of hours of training completed per week (Table 6-1). Overall, participants within the running and cycling groups can be described as well-trained athletes and provide a good opportunity for comparison between groups.

Table 6-1 Participant training habits and time trial performance

Variable	10-km Run (*n* = 11)		25-km Cycle (*n* = 11)	
	Mean ± SD	**Range**	**Mean ± SD**	**Range**
VO_2max (mL·kg·min^{-1})	56 ± 4	(49 – 62)	59 ± 8	(41 – 67)
Training experience (years)	7.7 ± 6.5	(1 - 22)	5.0 ± 4.9	(1 - 15)
Weekly distance (miles)	36.8 ± 11.1	(25 - 60)	114.5 ± 41.8**	(60 - 170)
Weekly frequency (days)	4.9 ± 1.4	(3 - 7)	4.0 ± 1.4	(2 - 6)
Training duration (hours)	7.1 ± 2.4	(4.0 - 10.5)	7.7 ± 2.1	(5.5 - 12)
TT times (min:sec)	46:15 ± 5:38	(39:53 - 49:02)	37:39 ± 2:30**	(34:03 - 42:16)

Significant difference between groups, * = P < 0.05; ** = P < 0.01). Data are means ± SD

6.4.1 Serum lubricin

Following exercise serum lubricin increased significantly irrespective of the exercise modality (Figure 6-3A); concentrations increased by 45.6% following cycling (baseline: 114.0 ± 41.8 ng/ml; immediately post-exercise: 166.0 ± 61.9 ng/ml, $P < 0.01$) and by 55.5% following running (baseline: 104.9 ± 29.5 ng/ml; immediately post-exercise: 163.1 ± 57.9 ng/ml, $P < 0.01$). Thirty minutes of seated rest resulted in a significant decrease towards pre-exercise values in both groups (cycling group: 112.0 ± 47.1 ng/ml; running group: 122.6 ± 49.1 ng/ml, both $P < 0.01$). The impact of exercise on serum lubricin is reinforced by the large effect size observed by the cycling (0.99) and running groups (1.27). There was no significant difference and only a small effect size (0.25) in baseline serum lubricin between cyclists (114.0 ± 41.8 ng/ml) and runners (104.9 ± 29.5 ng/ml). Sensitivity analysis did not alter the primary findings and indicated that any difference between baseline lubricin of cyclists and runners may be minimal.

6.4.2 Serum COMP

Serum COMP concentration increased in both groups following exercise (Figure 6-3B); however, this increase was significant following cycling only (baseline: 693.9 ± 189.0 ng/ml; immediately post-exercise: 916.4 ± 228.1 ng/ml, $P < 0.01$). Although non-significant ($P = 0.14$), the increase in serum COMP following running (baseline: 804.1 ± 259.3 ng/ml; immediately post-exercise: 918.1 ± 290.7 ng/ml) represented a moderate effect size (0.41). In a similar manner to lubricin, both groups demonstrated a significant return toward pre-exercise values following 30 minutes of seated rest (cycling group: 649.5 ± 176.2 ng/ml; $P < 0.01$, running group 752.6 ± 244.8 ng/ml; $P < 0.05$). No significant differences at baseline between the cycling and running group were revealed. However, mean baseline COMP tended to be 15.9% greater (moderate effect size, 0.48) in runners compared with cyclists ($P = 0.27$). Sensitivity analysis found serum COMP to be significantly increased following running ($P < 0.05$) as well as cycling ($P < 0.01$); this also indicated that differences in baseline serum COMP between runners and cyclists may be minimal.

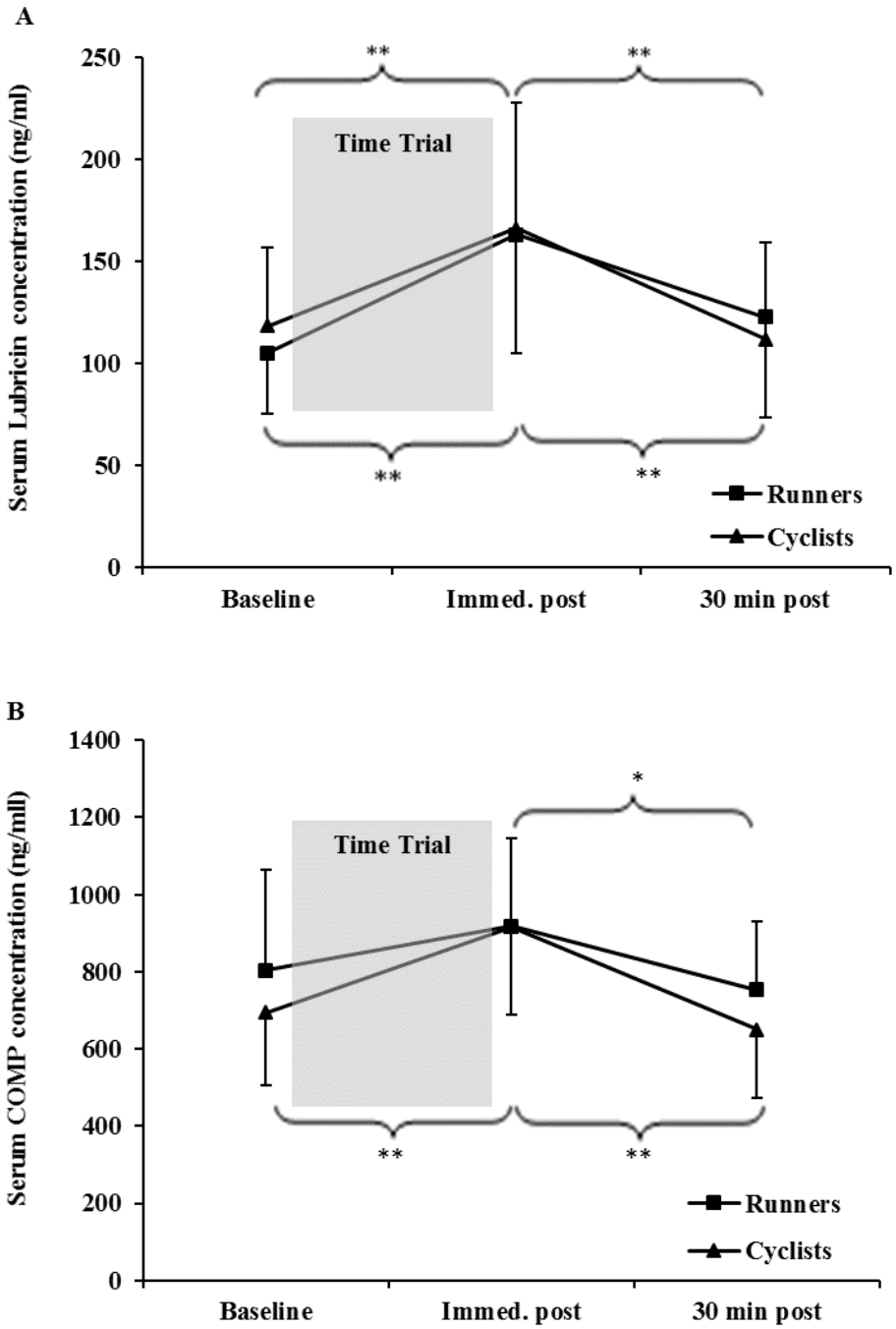


Figure 6-3 Mean A) serum lubricin and B) COMP concentrations, pre-exercise, immediately post, and 30 minutes post a 10-km running or a 25-km cycling time trial. * and ** = significant difference over time at $P < 0.05$ level and $P < 0.01$ level, respectively. Significance marked above data line represents cycling group and below represents running group. Data are means ± SD

6.4.3 Cartilage thickness, deformation and recovery

Cartilage thickness following running did not significantly change (all $P > 0.05$) at the intercondyle notch (baseline: 2.02 ± 0.47 mm; immediately post-exercise: 2.14 ± 0.48 mm), medial condyle (baseline: 2.15 ± 0.23 mm; immediately post-exercise: 2.09 ± 0.49 mm) or lateral condyle (baseline: 2.05 ± 0.21 mm; immediately post-exercise: 1.94 ± 0.34 mm). Likewise, there was no significant change (all $P > 0.05$) in cartilage thickness following cycling at the intercondyle notch, (baseline: 2.27 ± 0.38 mm; immediately post-exercise: 2.38 ± 0.63 mm) medial condyle (baseline: 2.04 ± 0.32 mm; immediately post-exercise: 2.02 ± 0.27 mm) or lateral condyle (baseline: 2.15 ± 0.31 mm; immediately post-exercise: 2.21 ± 0.28 mm) (Figure 6-4). In addition, following running and cycling the 30 minutes of seated rest provided did not significantly change intercondyle, medial or lateral femoral cartilage thickness. There was also no significant difference between baseline femoral cartilage thickness of runners and cyclists at the intercondyle notch, medial condyle and lateral condyle cartilage thickness (all $P > 0.05$). The greatest between group difference in baseline cartilage thickness was observed at the intercondyle notch (running group: 2.02 ± 0.47 mm: cycling group 2.27 ± 0.38 mm) which represented a moderate effect size (0.58).

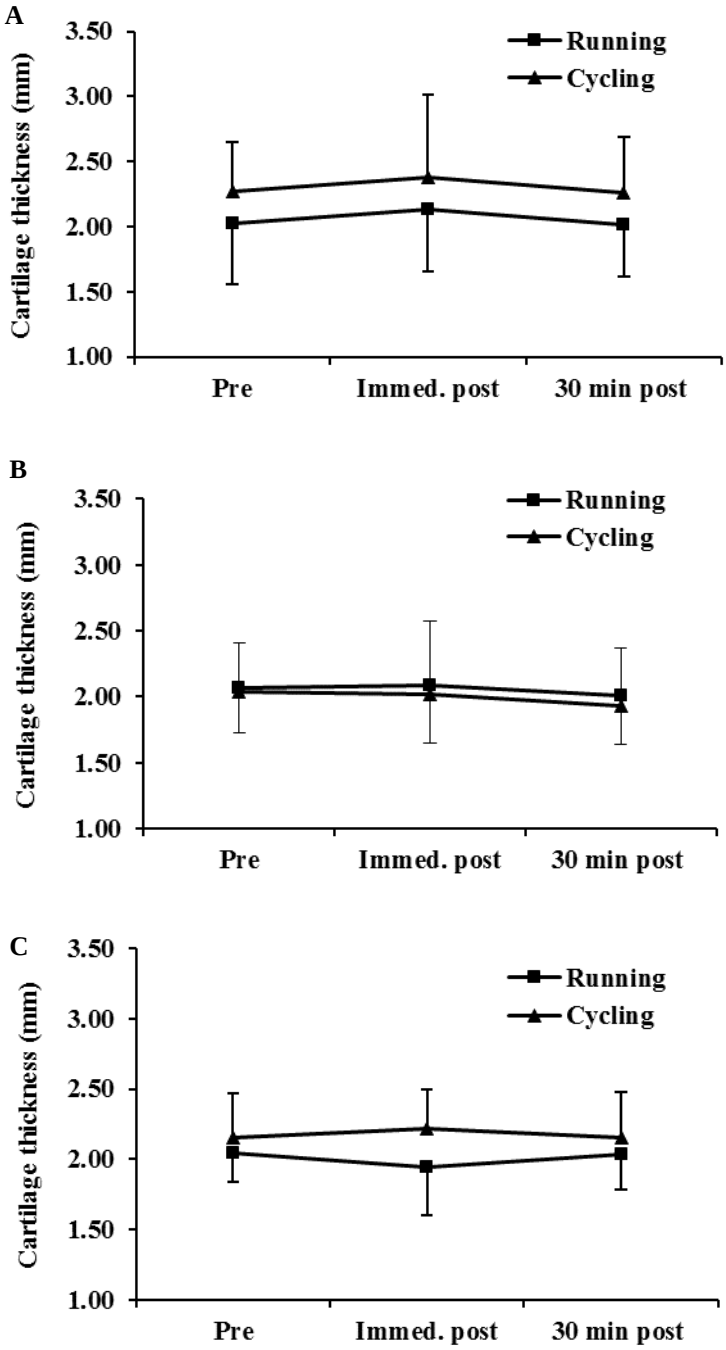


Figure 6-4 Mean cartilage thickness (mm) pre-exercise, immediately post, and 30 minutes post a 10 km running or a 25 km cycling time trial for a) intercondyle notch, b) medial condyle, and c) lateral condyle. Data are means ± SD

6.4.4 Correlation analysis

Correlation analysis was performed for the full sample (n =22), with very few correlations observed between outcome measures (Table 6-2). Advancing age was associated with baseline reduced cartilage thickness at the lateral condyle ($r = -0.650$, $P < 0.01$) and the number of previous training years was associated with increased cartilage thickness at the intercondyle notch ($r = 0.481$, $P < 0.05$). In addition, height was associated with the serum COMP response to exercise ($r = 0.573$, $P < 0.01$).

Table 6-2 Correlations between physical / exercise / training related parameters and parameters of joint function

Parameter	COMP baseline	Δ COMP	Lubricin baseline	Δ Lubricin	Baseline cartilage thickness		
					Notch	Medial	Lateral
Age	0.177	-0.130	0.010	0.271	-0.249	-0.064	-0.650**
Height	0.286	-0.573**	0.121	-0.343	0.003	0.263	0.334
Weight	0.325	-0.292	0.079	-0.064	0.272	0.012	0.114
BMI	0.268	0.134	-0.022	0.150	0.286	-0.066	-0.124
Body fat %	0.163	0.193	0.049	0.136	0.039	-0.205	-0.259
VO_2max	-0.198	-0.140	-0.169	-0.202	0.158	0.326	0.298
Time trial time	0.104	-0.141	-0.176	0.062	-0.236	0.112	-0.255
Training years	0.316	-0.207	0.021	0.350	0.481*	0.323	0.093
Training miles	-0.008	0.160	0.011	0.128	0.242	-0.230	0.225
Training frq / week	0.138	-0.182	-0.151	-0.386	-0.165	0.037	0.088

Δ, change from baseline to immediately post exercise; frq = frequency; (* $P < 0.05$; ** $P < 0.01$)

6.5 Discussion

This is understood to be the first study that has explored the effect of acute exercise on serum lubricin in humans. This study demonstrated that an acute bout of vigorous exercise results in an increase in both serum lubricin and COMP concentrations. In contrast to the serum biomarkers, femoral cartilage thickness was unaffected by vigorous exercise. In relation to the modality of exercise, the increase in serum lubricin following vigorous exercise was comparable for both runners and cyclists. However, unlike serum lubricin, the magnitude of change in serum COMP was less following running compared to cycling. The current study also demonstrates that serum lubricin, serum COMP, and femoral cartilage thickness do not significantly differ at baseline between runners and cyclists. Furthermore, there were no significant correlations between femoral cartilage thickness and either serum biomarker.

The increase in serum lubricin and COMP following acute vigorous cycling and running exercise in healthy humans in the current study are consistent with reports of increased PRG-4 and COMP production in response to mechanical load in cartilage explants and animal models (Abusara et al. 2013; Nugent-Derfus et al. 2007; Piscoya et al. 2005). The increase in serum COMP in the present study is also consistent with several studies that have previously demonstrated that serum COMP increases following exercise (Kersting et al. 2005; Neidhart et al. 2000; Niehoff et al. 2011). The increases in serum lubricin and COMP observed in the present study may reflect the release into the synovial fluid and circulation because of exercise-induced loading of the knee articular cartilage. However, the release from other joint sources such as the synovium, tendons, ligaments and bone, as well as non-joint sources cannot be discounted.

The elevation in serum lubricin and COMP concentration may be a result of the release of cartilage components into the synovial fluid and circulation in response to exercise. However, the exact mechanisms contributing to the increase in biomarker concentrations and the movement from the extra-cellular matrix into the bloodstream remains unclear. With regard to serum COMP, elevated levels within the circulation in response to loading may reflect increased cartilage turnover (Saxne et al. 1992), potential tissue damage (Neidhart et al. 2000), or an exercise-induced increase in the transport/removal from the joint into the blood (Helmark et al. 2012). In addition, acute increases in serum biomarkers conceivably may reflect the integrity of the joint. Importantly, joint movement is considered an important

factor in increasing intra-articular pressure and possibly contributes to the movement of cartilage constituents into the circulation (Levick and McDonald, 1995), thus supporting the exercise-induced increase observed in the present study.

Given the role of lubricin in reducing the coefficient of friction within the joint (Jay et al. 2007a), the acute increase in serum lubricin may reflect a temporary increase in joint lubrication following exercise. This may also explain why exercise has previously been shown to improve symptoms such as knee joint stiffness and pain among individuals with joint disease (O'Reilly et al. 1999). Interestingly, several *in vivo* animal studies have recently demonstrated that lubricin expression is attenuated under conditions associated with high levels of joint loading, including repeated high intensity exercise and with abnormal loading (Abusara et al. 2013; Elsaid et al. 2012; Ni et al. 2012). Therefore, due to the high impact loading associated with running loads compared to cyclists it was postulated that runners might demonstrate reduced resting lubricin concentrations. However, in contrast to the working hypothesis, no differences were observed between exercise modalities. In addition, there was no correlation between baseline lubricin and any parameter of training history, including miles, frequency or duration of training; however, this could be related to the homogenous sample, which was similar in age, fitness level and training habits.

In terms of serum COMP, increases are typically associated with a 'load-dependent' change, with the greatest increase following extensive running (Neidhart et al. 2000; Niehoff et al. 2011). In the present study serum COMP in the running group increased by +14.2% with acute exercise and was comparable to the +14.7% increases reported following a 1-hour training run (Kersting et al. 2005), and +15.0% increase following 31 km of marathon running in trained endurance runners (Neidhart et al. 2000). However, the current finding that serum COMP increased more following cycling versus running (+32.1% vs +14.2%) is somewhat surprising given that cycling is associated with lower tibiofemoral forces than running (Kutzner et al. 2012). Besides the magnitude of joint load, loading frequency has been suggested as an important factor in COMP release (Piscoya et al. 2005). However, given that the average cadence of cyclists and the typical stepping rate of recreational runners are similar, it appears unlikely that loading frequency was responsible for the results in the present study. Furthermore, the impact of loading frequency on the acute increase of serum COMP is not a universal finding. Thirty minutes of two different impact-loading conditions, i.e. running (high frequency, low amplitude) and drop landing (low frequency, high

amplitude), have also been shown to result in a very similar serum COMP response (Niehoff et al. 2011).

Baseline serum COMP concentrations did not significantly differ between runners and cyclists, and both groups were well within previously reported upper normal limits (<5000 ng/ml) (Saxne et al. 1992). Previously, baseline concentrations in marathon runners have been shown to be greater than the normal limit of 5000 ng/ml and comparable to the elevated baseline levels previously reported in individuals following joint injury and in patients with OA (Catterall et al. 2010; Neidhart et al. 2000). The baseline serum COMP concentrations found in the current healthy cohort are lower than previously reported in clinical populations, such as OA and RA (Law et al. 2015; Neidhart et al. 2000). This difference suggests that the trained runners and cyclists have a healthy cartilage turnover and limited, if any, cartilage degeneration. The greater levels of serum COMP previously reported in marathon runners (Neidhart et al. 2000) may result from differences such as increased training history and/or the onset of early degenerative joint change, or perhaps methodological differences such as the inclusion criteria and exercise restrictions before the blood sampling. The large degree of variation that exists in the reported baseline values indicates caution is warranted when comparing between studies.

The present study is understood to be the first to utilize ultrasonography to assess femoral cartilage thickness at baseline and following exercise in a group of healthy well-trained runners and cyclists. In contrast to the initial hypothesis, the results of the current study demonstrate that neither vigorous running nor vigorous cycling resulted in significant deformation of the femoral cartilage. In the present study, there was no significant deformation in cartilage thickness following either acute running or cycling. In contrast, running for a period of 30 minutes has previously resulted in cartilage thickness decreases at the femur (4%-8%) and tibia (0%-12%) in both marathon runners and sedentary controls (Mosher et al. 2010). Significant cartilage thickness deformation has also been demonstrated at the medial femoral condyle (-2.6%) following 30 minute of running in a sample of healthy young adults (Niehoff et al. 2010). The difference between the exercise-induced decrease in cartilage thickness observed in the studies and the present study might relate to the use of US to measure cartilage thickness compared to MRI. Although ultrasonography is valid, reliable (Naredo et al. 2009) and reduces the time-delay in measurement following exercise, MRI not only increases the ability to detect small changes following exercise due to a greater

measurement precision, but also provides the opportunity to measure individual cartilage plates as well as total cartilage volume compared femoral cartilage thickness alone. Nonetheless, differences in the frequency and amplitude of mechanical loading may also influence cartilage deformation (Niehoff et al. 2011). Furthermore, it is also possible that differences relate to potential adaptations in the biomechanical and/or mechanical properties of articular cartilage in response to chronic mechanical loading (Van Ginckel et al. 2010); this may explain potential differences between trained and less trained individuals.

The assessment of baseline femoral cartilage thickness using US has been largely limited to patient cohorts, including for example, individuals with OA (Tarhan et al. 2003), Meniscal injury (Akkaya et al. 2013b), spinal cord injury (Kara et al. 2013), and pes planus (or flatfoot) (Öztürk et al. 2015). Furthermore, there remains a paucity of studies using ultrasonography to assess formal cartilage thickness in healthy cohorts. Despite this, one recent study using ultrasonography demonstrated that healthy men whom regularly engage in exercise have thicker femoral cartilage than individuals who do not (Özçakar et al. 2014). Interestingly, in the present study, baseline cartilage thickness did not significantly differ between exercise modalities, though a moderate effect size indicated that runners may perhaps have reduced cartilage thickness at the intercondyle notch compared to cyclists. The effect of 3 months (Cotofana et al. 2010) and 6 months (Hinterwimmer et al. 2013) running training on patellar, femoral and tibial cartilage volume and thickness found limited change, thus suggesting that running training is well tolerated. Furthermore, marathon runners have previously been shown to have significantly greater femoral cartilage thickness compared to sedentary controls (Mosher et al. 2010). Therefore, weight-bearing exercise, such as running, appears to be both well tolerated and potentially positive for cartilage morphology compared to sedentary behaviour. However, the slightly greater cartilage thickness observed in the cycling group suggests that low impact non-weight bearing exercise, such as cycling, could perhaps provide a slightly superior loading stimulus for cartilage thickness at the weight-bearing femoral intercondyle notch. A larger sized study is required to determine whether cycling is more favourable for the knee joint compared with running. Interestingly, the current study also demonstrated a positive correlation between cartilage thickness and the number of training years across both runners and cyclists. This finding seemingly provides further evidence for the benefits of regular training on cartilage morphology and supports the previous work of Özçakar and colleagues (2014).

Despite the current encouraging research, it must be acknowledged that this study does have some limitations. Although joint structures are understood to be a primary source of lubricin and COMP, both are also expressed in several different tissues and neither are produced exclusively within the knee joint. Further work is therefore required to determine whether serum markers, particularly with exercise, reflect a functional or structural change at the knee joint. Research is also warranted to understand the movement of these biomarkers from the joint cavity into the circulating blood. Moreover, the ELISA assay used in the present study to represent serum lubricin detects not only the lubricin protein, but also several post-translational modifications of the PRG-4 gene including, superficial zone protein, megakaryocyte stimulating factor and hemangiopoietin. Therefore, it cannot be discounted that exercise-induced increases may also reflect an increase from other organs because of metabolic exercise-induced stressors. Also, as mentioned previously, shortcomings of US compared to MRI are also acknowledged (as mentioned previously). A study utilising greater variation in athletic ability and the type of exercise training, as well as including a control group may also help establish if differences exist between weight bearing and non-weight bearing exercise.

6.6 Conclusion

The current study suggests that an acute bout of either running or cycling stimulates an increase in cartilage metabolism and may also offer joint protection through lubricin provoked joint lubrication. Given the role of lubricin in maintaining joint function, it is suggested that the increase in serum lubricin may indicate a potentially therapeutic and protective benefit at the joint level. Finally, serum lubricin, serum COMP, and femoral cartilage thickness does not significantly differ between healthy trained runners and cyclists; the reported values would seemingly indicate a natural variation in a group of healthy trained individuals. Future studies should progress these encouraging and novel findings by investigating a less homogenous sample, as well as developing a greater mechanistic understanding regarding the effect of exercise on serum biomarkers in humans. These will be investigated further in Chapter 7.

Study contributors:

Harry Roberts, A/Prof Jeanette Thom and Dr Jonathan Moore conceived and designed study; Harry Roberts and Claire Griffiths-McGeever collected raw data; Harry Roberts and Dr Matt Fortes completed the preparation and analyses of blood samples; Harry Roberts completed all data analyses and prepared chapter.

7 CHAPTER 7. The effect of aerobic walking and lower body resistance exercise on serum COMP and hyaluronan, in both males and females

7.1 Abstract

PURPOSE: To compare the serum COMP and HA response to walking (high repetition loading) and resistance training exercise (low repetition loading) in both male and females.

METHODS: 15 males (age: 28 ± 6 years; BMI: 24 ± 2; mean ± SD) and 15 females (age: 26 ± 4 years; BMI: 23 ± 2) completed both a 40-minute walk at 80% HRmax and a 40-minute lower body resistance training protocol, separated by a minimum of 48 hours. Serum COMP and HA were determined by ELISA at rest, immediately after, and 30 minutes after each exercise intervention. Resting femoral cartilage thickness was also measured using ultrasonography.

RESULTS: Serum COMP concentrations increased following walking (28.9 %; $P < 0.001$) and resistance training exercise (26.0 %; $P < 0.001$), prior to returning towards baseline concentrations after 30 minutes of rest. The response did not differ for modality or gender. However, males did demonstrate higher concentrations of serum COMP compared to females at all time points (all, $P < 0.001$). In contrast, serum HA did not change following either modality of exercise. However, females demonstrated higher HA concentrations at all time points compared to men, which was significant both pre-exercise ($P = 0.006$), and immediately post exercise ($P = 0.033$). Finally, following adjustment for body size, femoral cartilage thickness was greater in men compared to women at the notch ($P < 0.001$).

CONCLUSION: Lower body resistance and walking exercise affect cartilage biomarkers similarly in healthy male and female individuals. Moreover, thicker femoral cartilage and higher baseline serum COMP concentrations in males does not appear to influence cartilage response to exercise loading.

7.2 Introduction

Understanding the influence of age and physical activity on cartilage structure and function is important to increase the knowledge of the potential risk and/or benefits associated with the pathogenesis of OA and other degenerative joint diseases. Consequently, this study attempts to address several research questions in relation to the impact of exercise modality and sex differences using both serum biomarkers as surrogate markers of the knee joint cartilage, as well as the direct measurement of femoral cartilage thickness.

In Chapter 5, it was demonstrated that markers associated with knee OA, including serum concentrations of COMP, HA and lubricin, as well as femoral cartilage thicknesses do not change greatly with age, or with increasing levels of physical activity in healthy adults. Instead, the results suggest that a healthy variation exists in these markers. Additionally, in Chapter 6 it was demonstrated that in a group of healthy well-trained runners and cyclists there appears to be no difference in cartilage thickness or serum COMP and lubricin. Interestingly, it was also demonstrated that an acute bout of weight bearing exercise (running) and non-weight bearing exercise (cycling) result in a comparable acute increase in markers associated with cartilage catabolism/metabolism and lubrication, which return to resting levels following 30 minutes of rest. These results indicate that there is minimal difference in aerobic weight bearing and aerobic non-weight bearing exercise. Thus leading to the understanding that this acute exercise response represents a normal healthy response to exercise. This exercise-induced response is in accordance with several previous studies that have also shown an increase in serum COMP following activities such as running and walking, which involve joint loading that are high in loading frequency but relatively low in loading amplitude (e.g. Mündermann et al. 2005; Mündermann et al. 2009; Niehoff et al. 2010; Celik et al. 2013; Denning et al. 2015). Interestingly, recent research has suggested that the magnitude of the serum COMP response to walking and running exercise is related to both joint mechanics and load frequency (Denning et al. 2016).

Whether knee joint loading through resistance training results in the same response as aerobic exercise such as walking or running remains an important question. There are currently only a few studies that have explored the effect of knee joint loading through high load and low repetition exercise on serum biomarkers and the findings have been mixed. Slow deep knee bends did not result in an acute increase serum COMP (Niehoff et al. 2010), while similarly,

in RA patients acute lower body resistance exercise involving 3 sets of 8 reps of did not result in a significant increase in serum COMP (Law et al. 2015). In contrast, drop jumps in healthy individuals have been shown to result in a significant increase in serum COMP (Behringer et al. 2014; Niehoff et al. 2011). Based on the said studies, increases following lower-body specific exercise could relate to the magnitude of joint loading as well as the studied population. To date, no study has explored the serum biomarker response to a typical lower-body resistance exercise in healthy individuals. Furthermore, it is understood that all the studies that have previously explored differences in joint loading protocols have been related to serum COMP only, and not serum HA, a major component of the connective tissue (Seebeck and Haima, 2013) that has previously been correlated with OA (Elliott et al. 2005) and is considered a marker of synovial inflammation and linked with cartilage degradation (Garnero et al. 2001).

Moreover, this study explores the impact of sex on both baseline and the exercise-response to exercise. Women have previously been shown to have both reduced femoral cartilage thickness (Özçakar et al. 2014) and lower levels of serum COMP compared to men (Jordan et al. 2003; Mündermann et al. 2005; Verma and Dalal, 2013). It has been suggested that differences in cartilage between men and women relate to a smaller body size and reduced overall cartilage (Ding et al. 2003). Crucially, women have a greater risk of knee injuries in comparison to men, while older women also have a greater risk of developing OA compared to men (Arendt and Dick, 1995; Felson et al. 1987). Women have also been shown to have different neuromuscular and biomechanical loading patterns compared to men, possibly influencing their susceptibility to injury and OA (Russell et al. 2006). Knee injury rates in women have also been associated with differences in sex hormone concentration (Slauterbeck et al. 2002), while increases in OA in older women are understood to coincide with an oestrogen reduction post-menopause (Sniekers et al. 2010).

Therefore, the primary aim of this study was to compare the effects of an acute bout of high load low frequency exercise with a low load high frequency bout of aerobic exercise. Secondary aims of this study were to determine whether sex influences cartilage thicknesses and serum biomarkers at baseline, as well as potential exercise-induced changes in these variables. In contrast to Chapter 5 and 6, this study focussed solely on serum COMP and HA, and did not include serum lubricin. Firstly, this decision based on the finding that serum lubricin responded in the same way as serum COMP to exercise (Chapter 6). In addition, an

improved understanding of the assay indicated that not only the lubricin protein, but also several post-translational modifications of the PRG-4 gene could be detected; thus suggesting that the exercise-induced increases may also reflect an increase from other organs. Likewise, femoral cartilage thickness was only assessed at baseline and not following exercise. Given that in Chapter 6, vigorous exercise did not result in any significant cartilage deformation, it was decided that the lower intensity activities employed in the present study would be less likely to identify a change in cartilage thickness.

Hypothesis 1: Acute lower body resistance exercise would result in an exercise-induced increase in serum concentration of COMP and HA

Specific objective 1: To test the first hypothesis, a blood sample was obtained via venepuncture pre-exercise, immediately following and 40 minutes of lower body resistance exercise training. Serum concentrations of COMP and HA were determined using commercially available ELISAs.

Hypothesis 2: The magnitude of change of serum biomarkers would be comparable following 40 minutes of walking compared to isolated lower body exercise

Specific objective 2: To test the second hypothesis, participants completed two experimental acute exercise protocols, each performed on a different day. One protocol consisted of lower body resistance exercise and the second consisted of a standardised aerobic walking protocol. Blood samples were obtained via venepuncture pre-exercise, immediately post, and 30 min post each exercise protocol. Serum concentrations of COMP and HA were subsequently determined using commercially available ELISAs. The differences between pre-exercise and post values were determined and compared between modalities.

Hypothesis 3: At baseline women would demonstrate reduced cartilage thickness and reduced levels of serum COMP and HA compared to men. We proposed that this difference at baseline would not remain once body size was taken into consideration as a covariate.

Specific objective: To test this third hypothesis a well-matched group of healthy men and women underwent a sonographic assessment of cartilage thickness and provided a resting blood sample, which were obtained following thirty minutes of seated rest. Serum concentrations of COMP and HA were determined using commercially available ELISAs.

Hypothesis 4: Both men and women would demonstrate a comparable serum profile in response to and following acute exercise.

To test this forth hypothesis a group of well-matched males and female completed two comparable acute exercise protocols. Serum concentrations of COMP and HA were determined using commercially available ELISAs.

7.3 Methodology

7.3.1 Participants

In total, thirty-five healthy participants were recruited for this study and commenced baseline testing. The aim of this recruitment was to enlist both a healthy group of male individuals and a healthy group of female individuals, which were well matched for age, BMI and activity history. Participants were targeted through word of mouth, poster advertisement, generic emails, and social media from the Bangor University community and the surrounding North Wales area. Details of the recruitment process through to study completion are outlined in Figure 7-1. The inclusion for entry to the study included being: (i) male or female (ii) aged between 18-40 years (iii) BMI of < 30 kg / m^2.

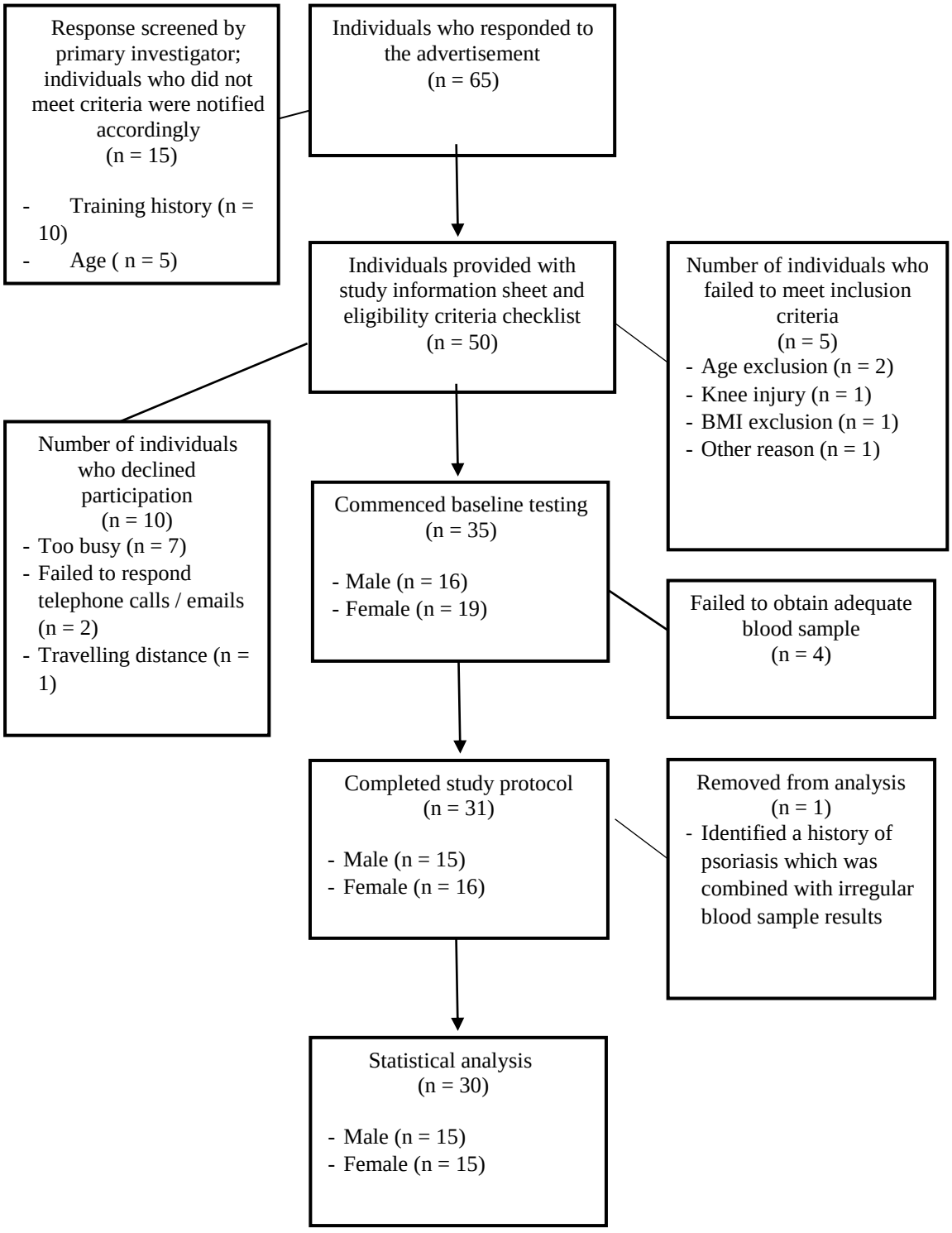


Figure 7-1 Participant flow diagram detailing participant recruitment, baseline testing and protocol completion

7.3.2 Experimental protocol

In this two group, randomised, crossover designed study, participants were required to visit SSHES, Bangor University on three separate occasions as outlined below in Figure 7-2.

Visit 1

During this initial visit, participants were given a full verbal explanation of all procedures and given the opportunity to ask questions, prior to completing both medical and physical activity questionnaires. Full detail regarding the IPAQ 7-day long questionnaire and the 12-month physical activity questionnaire used in this study are outlined in Chapter 3 and can be viewed as Appendix A and B, respectively. Following a period of 30 minutes of seated rest, femoral cartilage thickness was assessed using ultrasonography (as specified in Chapter 3) before the measurement of body mass and height. Participants subsequently completed a submaximal treadmill (HPcosmos Mercury 4 Med, Nussdorf-Traunstein, Germany) walking protocol to estimate maximum oxygen uptake (VO_2max) (Ebbeling et al. 1991). This protocol consisted of an initial 4-minute walk at a brisk but comfortable walking speed (3 and 4.5 mph) with heart rate within 50-70% of HRmax. If heart rate was not within the required range after the first minute of exercise the speed was adjusted accordingly. Following the initial 4-minute period, the gradient was increased to 5% for the subsequent 4 minutes. Heart rate and RPE was monitored throughout. In addition, participants who did not reach an intensity of 80% HRmax during this submaximal test were required to complete further incremental walking exercise using the treadmill until 80% HRmax or an RPE of 15 was achieved. This allowed the determination of the appropriate exercise intensity (walking speed and incline) for the walking exercise intervention. Finally, participants completed an 8-repetition maximum (RM) test of the leg press, leg extension and leg curl exercises (Whaley et al. 2006). This 8-RM test allowed the 1-RM to be accurately estimated using a regression equation (Brzycki, 1993). The resistance training protocol followed ACSM guidelines for muscle strength training by utilising 80% of the 1-RM for both the leg press, leg extension, and leg curl exercises. All exercises were performed in the departmental laboratory using commercially available leg press machine (HUR Main Line Leg Press 3540) and seated leg extension/curl weights machines (Powersport International Limited, 1986).

Visit 2 and 3

Visit 2 and 3 consisted of the exercise trials. Importantly, the order in which the exercise bouts were randomized. On arrival to the laboratory, participants were required to rest for 30 minutes before providing a baseline blood sample. Participants subsequently completed either an aerobic walking protocol, or a lower body resistance exercise protocol. Upon immediate completion of the exercise trial, a second blood sample was obtained. Lastly, following 30 minutes of seated rest post exercise a final blood sample was obtained.

Exercise intervention

The exercise protocols were designed to offer an aerobic and resistance training stimulus that was matched for time. Importantly, this study adopted a pragmatic approach that aimed to assess the impact of 'real-world' exercise sessions on markers associated with knee joint cartilage. The aerobic walking protocol was designed to offer a low load, high frequency stress. While in contrast, the resistance training protocols offered a high load, low frequency stress. Additionally, heart rate was assessed at regular intervals throughout both exercise protocols, while blood lactate was assessed at rest and following completion of each exercise intervention. Heart rate and blood lactate were used to monitor the stress associated with the activity and to aid the comparison of each activity. Blood lactate was assessed via capillary blood sampling (5 ul), collected from the fingertip and immediately analysed using a portable lactate analyser (LactatePro, Arkray, Japan). Prior to each test, the lactate analysers were calibrated in accordance with the manufacturers guidelines.

Walking protocol

The walking protocol consisted of 40 minutes of treadmill walking exercise. The exercise intensity was derived from the walking protocol conducted during the first visit to the department. As appropriate, the speed and incline was adjusted throughout to ensure all participants maintained an intensity as close to 80% HRmax as possible.

Resistance training protocol

This session included 40 minutes of lower-body resistance training. This training aimed to specifically target muscles around the knee joint, optimising high load, low frequency loading of the knee. In total, five exercises utilised, the leg press, leg extension, leg curls, squats and alternate lunges. Each resistance machine exercises (leg press, leg extension, and leg curl) consisted of one set of 15 repetitions with half-load, prior to completing three sets of

eight repetitions at 80% 1-RM. Similarly, both the squat and alternate lunge exercises, involved completing one set of 15 body weight repetitions, prior to completing three sets of eight repetitions using dumbbells of 10% body weight. A minimum of one minute of rest was provided between sets. All participants were informed to complete the exercises in a controlled manner with correct exercise form with an emphasis on limiting the aerobic exercise response.

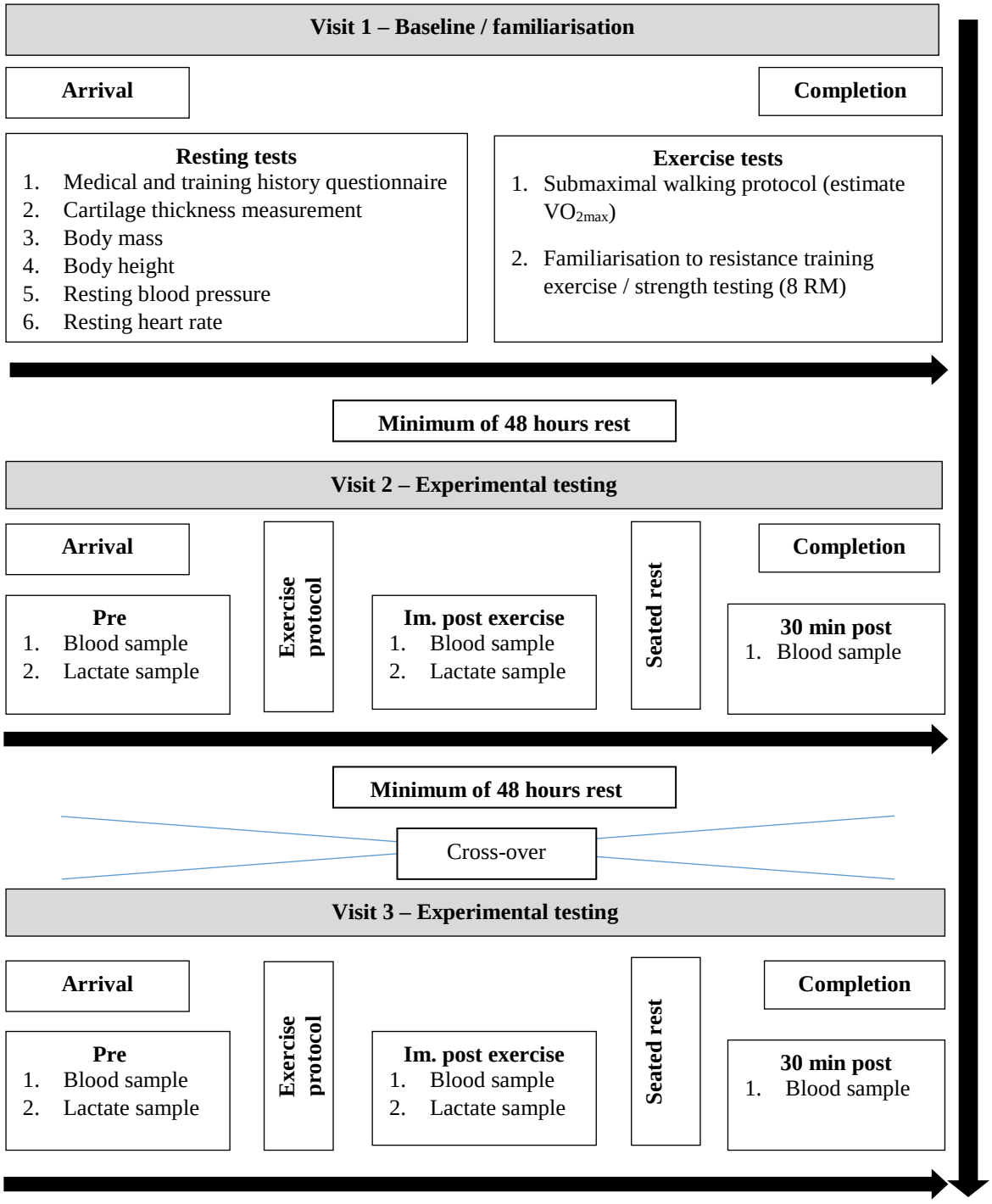


Figure 7-2 A schematic overview of the study protocol.

7.3.3 Statistical analysis

Statistical analyses were performed utilising statistical analysis software [SPSS for Windows version 20.0 (SPSS, Chicago, IL, USA)]. A three-factor mixed design was used to assess the effect of exercise intervention (walking vs resistance training), sex (male vs female) and time (pre, immediately post exercise, and 30 minutes post exercise), on each dependent variable (serum COMP and serum HA). Significant interactions and/or main effects were analysed post hoc using Bonferroni-corrected t-tests where appropriate. Independent sample t-tests were used to assess differences between males and females. Independent sample t-tests were also conducted to determine whether differences in mean cartilage thickness exists between male and female participants at each location (right intercondyle notch, lateral condyle, medial condyle). As appropriate analysis of covariance (ANCOVA) analyses was subsequently used to adjust for differences in body size. For this analysis, a composite variable reduced from weight and height was used. Normality of data was explored by visual inspection of Q–Q plots and through analysis of the model's residuals and outliers were removed as necessary. All figures and tables are presented as mean ± SD, with statistical significance set as ($P < 0.05$).

7.4 Results

Thirty participants (male n = 15; female n = 15) matched for age and BMI were included within the analyses. Anthropometric, physical characteristics training habits for both groups are shown in Table 7-1. Unsurprisingly, males were significantly taller and heavier than female participants. Familiarisation tests also identified that males had both a greater estimated VO_2max and absolute lower-body muscle strength. Training habits were comparable between groups for the number of training years, average number of days, average number of hours completed per week, physical activity over the last 7 days (7 day IPAQ) and physical activity over the last 12 months. Overall, participants studied can be described as healthy, recreationally active males and females that provide a good opportunity for comparison between groups.

Table 7-1 Baseline anthropometric, physical characteristics and exercise habits of participants in the two groups

	Male		Female	
Variable	**Mean +/- SD**	**Range**	**Mean +/- SD**	**Range**
Age (years)	28±6	19-40	26±4	20-33
Height (metres)	1.77±0.04	1.72-1.84	1.67±0.07**	1.51-1.78
Weight (kg)	77±7	62-88	64±9**	40-82
BMI (kg/m^2)	24±2	20-27	23±2	18-26
Estimated VO_2max	56±4	50-65	48±3**	44-54
Leg press (8RM)	199 ± 32	150-250	150±31**	110-230
Leg extension (8RM) (kg)	36±13	20-65	21±7**	10-40
Leg curl (8RM) (kg)	17±7	5-35	10±5**	5-25
Lifetime training experience (years)	11±7	2-29	11±7	2-20
Weekly frequency (day/week)	3±2	0-7	4±2	0-6
Training duration (hr/week)	3±3	0-10	5±3	0-12
7 day IPAQ (MET min/week)	4096±3701	777-14838	2952±2005	1152-8748
12 month physical activity index	8.4±1.0	6.9-10.1	7.7±1.4	5.1-9.3

MET = metabolic equivalent; Significant difference between groups (* P < 0.05; **P < 0.01). Data are means ± SD

7.4.1 Serum COMP

Mean serum COMP significantly increased from baseline following both modalities of exercise. Following walking, serum COMP concentration increased by 28.9% (baseline: 490.3 ± 200.2 ng/ml; immediately post exercise: 631.8 ± 223.4 ng/ml) and following resistance training, serum COMP concentrations increased by 26.0% (baseline: 501.8 ± 180.0 ng/ml; immediately post exercise: 632.5 ± 196.0 ng/ml). Following a period of 30 minutes of seated rest, serum COMP concentrations returned towards baseline (walking: 518.6 ± 210.8 ng/ml; resistance training group: 473.3 ± 169.1 ng/ml) and were no longer significantly different from baseline concentrations in both interventions ($P > 0.05$). There was no significant difference at baseline, in the exercise response, or the decrease following exercise between the walking and resistance training interventions (

Figure **7-3**). Similarly, the change in serum COMP concentration over time was comparable between males and females (

Figure **7-3**). However, serum COMP concentrations were higher in males at baseline (595.0 vs 395.4 ng/ml), immediately post exercise (751.6 vs 517.6 ng/ml) and 30-minutes post exercise (591.2 vs 400.7 ng/ml) (all, $P < 0.001$).

7.4.2 Serum HA

Mean serum HA did not significantly change following either walking or resistance exercise (all, $P > 0.05$). Furthermore, there was no difference over time between males and females. However, mean serum HA concentrations were higher in females compared to males at baseline (37.7 vs 26.2 ng/ml, $P = 0.006$), immediately post exercise (38.0 vs 28.2 ng/ml, $P = 0.033$) and at 30-minutes post exercise (36.0 vs 28.2 ng/ml, $P = 0.107$) (Figure 7-4).

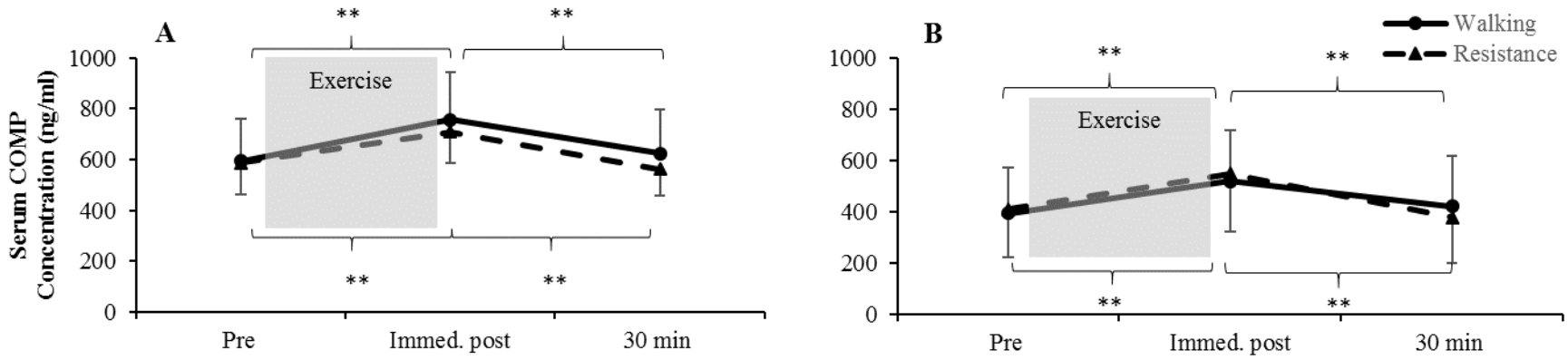


Figure 7-3 Mean serum COMP concentration, pre-exercise, immediately post, and at 30 min post 40 min of walking or 40 mins of resistance training exercise in A) males and B) females. * and ** = significant difference over time at P < 0.05 level and P < 0.01 level, respectively. Significance marked above data line represents walking group and below represents resistance training group. Data are means ± SD

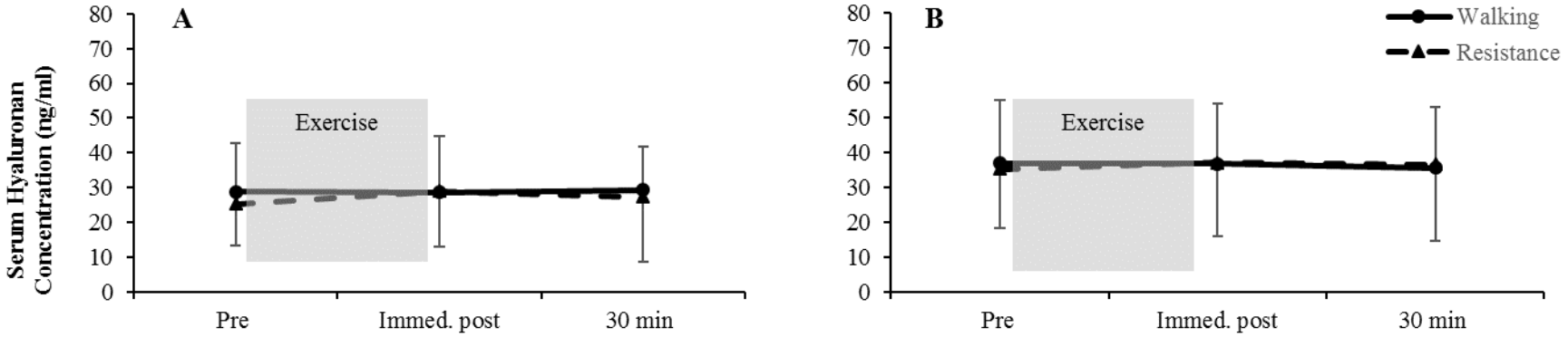


Figure 7-4 Mean serum HA concentration, pre-exercise, immediately post, and at 30 min post 40 min of walking or 40 mins of resistance training exercise in A) males and B) females. Data are means ± SD

7.4.3 Cartilage thickness

The assessment of cartilage thickness revealed that males had significantly thicker cartilage compared to females at the intercondyle notch, medial condyle and lateral condyle (Table 7-2). The greatest mean difference in cartilage thickness was at the intercondyle notch, followed by the medial and lateral condyles (Table 7-2). Furthermore, there was a significant difference between males and females in mean cartilage thickness at the intercondyle notch [F (1,25) = 23.497, P = 0.001] whilst adjusting for body size using a composite variable that considered both the height and weight of participants. ANCOVA analyses were not completed for the lateral and medial condyle due to violations in key test assumptions.

Table 7-2 Mean cartilage thickness (mm) in both males and females

	Male		**Female**	
Variable	**Mean +/- SD**	**Range**	**Mean +/- SD**	**Range**
Cartilage thickness (mm)				
Notch	2.50±0.25	2.04-2.98	1.91±0.25 **	1.57-2.47
Lateral	2.18±0.22	1.79-2.49	1.82±0.28 **	1.31-2.29
Medial	2.18±0.37	1.52-2.73	1.74±0.13 **	1.55-1.93

Significant difference between groups (* P < 0.05; ** P < 0.01). Data are means ± SD

7.4.4 Blood lactate

Blood lactate concentrations significantly increased following resistance training exercise (pre:1.7 vs post: 4.3 mmol/L, P < 0.001). In contrast, despite an increase following the aerobic walking exercise protocol (pre: 1.6 vs 2.2 mmol/L) this did not reach significance (P = 0.07). No difference was observed between sexes.

7.4.5 Heart rate

The average intensity of participants completing the walking protocol was 76% of age predicted HRmax. In contrast, the average intensity of participants completing the resistance training protocol was 55% HRmax.

7.5 Discussion

This study demonstrated for the first time that acute walking and resistance exercise result in a similar temporary increase in serum COMP. This study is also the first to directly establish that the serum COMP response to exercise is unaffected by sex. However, in contrast to the first and second hypotheses, serum HA remained unaffected by either bout of exercise. Moreover, although sex was found to be unrelated to the exercise response, men were found to have higher level of serum COMP, lower levels of serum HA and revealed thicker femoral cartilage at all locations compared to women.

Several studies have previously demonstrated that walking results in an acute increase in serum COMP (Celik et al. 2013; Denning et al. 2016; Mündermann et al. 2005). The exact mechanism contributing to the increase in serum COMP is unknown, however it is understood to be a physiological response that reflects increased healthy cartilage turnover or metabolism, rather than cartilage degradation. It would seem unreasonable to expect cartilage damage from acute walking or resistance exercise in a group of healthy individuals. The increase in serum COMP following walking in the present study (+28.9%) was greater than previously reported by Mündermann et al. (2005) and Denning et al. (2016) following walking, +9.7% and +5.27%, respectively. This difference may be due to a shorter bout of exercise (Mündermann et al. 2005) or have been self-paced bouts of walking (Denning et al. 2016). However, If the increase in serum COMP is assumed to be linear, walking in the present study was associated in an increase of 7.2% / 10 min of exercise, compared with 3.2% / 10 min and 3.4% / 10 min in studies by Mündermann et al. (2005) and Denning et al. (2015), respectively. The increase in serum COMP following walking in the present study (+28.9% or +7.2% / 10 min of walking) in the present study was comparable to the increase in serum COMP following a bout of vigorous cycling (+32.1% or +8.4% per 10 min of cycling) and higher than vigorous bout of running (+14.2% or +3.1% / 10 min of running) in trained individuals (see Chapter 6). A greater increase following walking compared to vigorous running was somewhat surprising given that the overall load following running was higher (exercise time: 40 min vs a 46 min (average); intensity of exercise: 76% vs 90.4% HRmax; distance covered: 4.2 vs 10 km). Another plausible possibility for this finding relates to the training status. For example, the participants in the present study were generally less trained than the individuals who participated in the experimental research in Chapter 6. Previous research has shown that exercise training may lessen the acute serum COMP

response to acute walking exercise (Celik et al. 2013), potentially by consolidating the cartilage matrix and consequently reducing release of COMP from the extracellular matrix and eventually into the circulation.

The disparity in the results between studies may also relate to body composition. BMI was previously related to thinner cartilage, particularly at the lateral condyle (Chapter 5). Previous studies have also demonstrated that body weight independently affects the increase in serum COMP to walking (Denning et al. 2015). Although BMI per se was no different between the walking and running groups in the present thesis, differences (i.e. a potentially greater) muscle mass and function in the more highly trained runners may be protective and contribute to the smaller serum COMP response to exercise. Further investigation is required into the effect of body composition and the potentially protective effects of muscle on the response of serum COMP to exercise.

The present study was the first to demonstrate that resistance exercise (low loading frequency, high loading amplitude) and walking (high in loading frequency, low in loading amplitude) result in a very similar serum COMP response. This supports previous research that found a similar serum COMP response when comparing running and drop jumps (Niehoff et al. 2011). Although loading frequency has been suggested as an important factor in COMP release (Piscoya et al. 2005), the present study provides evidence to suggest that in healthy individuals, cartilage responds in a very similar manner to a lower loading frequency when the magnitude of loading is increased. Moreover, loading frequency has previously been associated with the duration of elevated serum COMP post exercise (Denning et al. 2016). However, in the present study, both modalities of exercise resulted in a very similar post exercise return towards baseline indicating that type of loading did not influence the duration of serum COMP elevation post exercise either.

In line with the third hypothesis, this study established that differences exist in baseline concentrations between males and females. In agreement with the present study, baseline serum COMP concentrations have been shown to be lower in females compared to men (Jordan et al. 2003). Differences may be related to increased joint size, or to increased total cartilage, meniscal and tendon size in men compared to women (Jordan et al. 2003). However, height, which is often considered a surrogate measure of skeletal size, is not always associated with serum COMP (Jordan et al. 2003; Chapter 6). Other factors, including

differences hormones and growth factors may also explain these sex differences. Although sex hormones have been implicated in an increased risk of knee injury (Slauterbeck et al. 2002) and knee OA (Boyan et al. 2013), little is known regarding the effect on cartilage biomarkers. However, studies have found that serum COMP is decreased in response to hormonal replacement therapy in postmenopausal women with RA (Forsblad d'Elia et al. 2004) and osteopenia/osteoporosis (Seo et al. 2012). These studies infer that sex hormones may alter cartilage biomarkers expression and thus warrant further investigation.

Moreover, also in line with the forth hypothesis, the serum COMP response to exercise did not differ between males and females. Therefore, despite differences in baseline cartilage thickness, there appears to be no functional difference in cartilage turnover or metabolism between males and females who are matched for aged and BMI, and whom have very similar levels of training history and fitness. This finding was unsurprising given that cartilage deformation behaviour between women and men has also been found to be similar (Hudelmaier et al. 2001).

The present study found no evidence of any exercise-induced change in serum HA in healthy individuals. A previous report by Engström-Laurent et al. (1987) also found no evidence of a change in HA following moderate intensity cycling, although a bout of heavy cycling exercise resulted in a modest increase in HA. In contrast, moderate acute exercise in patients with RA elicited a large increase in serum HA (Engström-Laurent and Hällgren, 1987). A greater exercise-related increase in RA patients was related to synovitis mass, suggesting that joint inflammation may be key in the synthesis and accumulation of serum HA. In a separate study, plasma HA has been shown to rise with exercise time and demonstrate an exponential increase with increasing exercise intensity in healthy individuals (Hinghofer-Szalkay et al. 2002). As with serum COMP, any exercise-induced change in serum HA in healthy individuals is understood to be due to a physiological response rather than a change in structure. Based on the available literature, it is possible that the exercise duration and intensity employed for the present study may be insufficient to increase serum HA, at least in healthy men and women that are likely to have low levels of joint inflammation. It is possible that difference in the exercise-response between serum COMP and HA relate to either a greater release of COMP from the joint, differences in the transport across the joint membrane and into the systemic circulation, and/or differences in the clearance of biomarkers by the liver and kidney. Moreover, the present study found that baseline concentrations were

similar to previously reported values in healthy individuals and lower than those reported in individuals with joint disease, including OA (Criscione et al. 2005; Wakitani et al. 2007) and RA patients (Engström-Laurent and Hällgren, 1987). Surprisingly, the present study found higher serum concentrations of HA in women compared to men. Serum HA has previously been shown to be influenced by various individual factors, including sex, with higher serum HA concentrations typically found in men compared to women (Elliott et al. 2005). There is no clear explanation for the higher HA concentrations observed in this female population.

As in Chapter 6, it is essential to acknowledge that it has yet to be determined whether increases in COMP following exercise reflect cartilage turnover (Saxne et al. 1992), tissue damage (Neidhart et al. 2000), or an increase in the transport/removal from the joint into the blood (Helmark et al. 2012). Moreover, a recent study found that increased serum COMP following exercise corresponded with a decrease in synovial fluid COMP (Hyldahl et al. 2016). This supports previous findings indicating that exercise facilitates the movement of COMP from within the joint into the circulation (Helmark et al. 2012), possibly due to an increase in intra-articular pressure (Levick and McDonald, 1995). Moreover, in relation to serum HA, the unaffected serum concentration may indicate that HA remained within the joint despite an increase in exercise. Given that HA is used as a therapeutic intervention for OA (Shimizu et al. 2010) and considered an important joint lubricant (Schmidt et al. 2007), this may actually be a positive finding. Although the knowledge within the area of biomarkers is constantly advancing, further studies are required in order to determine how the response of serum biomarkers to loading reflects changes at the joint level.

The present study provides some new insights into the effect of exercise modality and sex on several cartilage biomarkers. However, it must also be acknowledged that this study does have some limitations. Despite attempting to provide a comparable exercise bout in relation to exercise time and intensity, resistance training result did result in a significant increase in blood lactate concentration, which was not observed following walking. This suggests that the metabolic stress associated with 40 minutes of resistance training may be higher than 40 minutes of walking. Moreover, in relation to the sex-differences observed in both serum COMP and serum HA, we must recognise that to date it remains unknown whether menstrual cycle phase, or use of oral contraceptives, significantly influences the serum concentration of these biomarkers. Furthermore, while we asked participants about comorbidities, we do not have objective data on liver and kidney function, both of which may particularly affect serum

HA levels. Crucially, despite strict methodological standardisation (as specified in the general methodology) and the use of the same commercially available ELISA kit across all experimental chapters, caution is required when comparing concentrations between experimental studies and when comparing absolute values with other published research studies.

7.6 Conclusion

The current study suggests that an acute bout of either walking or resistance exercise stimulates an increase in cartilage metabolism. This study also provides evidence to suggest that these exercise modalities, which comprise of markedly different loading patterns, effect the cartilage in a very similar manner and do not differ between sex. Moreover, the return of serum COMP following 30 min of rest suggests that the effect on joint tissues is temporary and well tolerated in healthy individuals. Importantly, this study indicates that 40 minutes of either walking or resistance exercise in healthy men and women, provides a comparable and healthy mechanical stimulus for joint cartilage. Given that joint loading is important for cartilage, including the avoidance of cartilage atrophy, walking and resistance exercise may be encouraged. To progress current understanding further, longitudinal studies should attempt to determine how cartilage is affected by regular long-term acute increases in serum biomarkers and whether the response to exercise changes with training. In addition, future studies should also attempt to provide additional detail of biomarker kinetics between synovial fluid and serum concentrations, particularly in relation to HA.

Study contributors:

Harry Roberts, A/Prof Jeanette Thom and Dr Jonathan Moore conceived and designed study; Harry Roberts collected raw data; Harry Roberts and Jason Edwards completed the preparation and analyses of blood samples; Harry Roberts completed all data analyses and prepared chapter.

8 CHAPTER 8: General Discussion

This aim of this thesis was to research several novel markers of knee joint health and to use these to explore how ageing and physical activity affects the knee. Given the crucial role of articular cartilage throughout the knee joint, it is important to be able to accurately and reliably assess knee cartilage morphology. Consequently, a primary aim of Chapter 4 was to determine the feasibility of using ultrasound to measure cartilage thickness by assessing the intra-session reliability and measurement error. Secondary aims of Chapter 4, together with Chapter 5, were to increase normative data for both femoral cartilage thickness measurements using US and serum biomarkers of knee cartilage in healthy adults. Moreover, as humans age, articular cartilage is understood to undergo substantial physiologic, mechanical, biochemical and functional changes that reduce its ability to overcome the effects of mechanical stress and injury (Luria and Chu, 2013). Therefore, in Chapter 5, a cross-sectional study was used to investigate whether healthy ageing is associated with early changes in femoral cartilage thickness and serum biomarkers of knee cartilage. Based on the understanding that regular exercise has several benefits for articular health, secondary aims were to explore whether physical activity, particularly the amount of physical activity, could moderate any age associated changes. In addition, measuring the acute responses of joint markers to exercise is also considered an important measure of cartilage composition and overall joint health. Thus, in Chapter 6 and 7, acute exercise interventions were used to explore the effect of different exercise modalities. Chapter 6 specifically explored the effect of weight bearing (running) and non-weight bearing (cycling) aerobic exercise on cartilage thickness and serum COMP and lubricin. Subsequently, Chapter 7, explored the effect of different types of joint loading i.e. walking (high frequency, low magnitude) exercise vs resistance training (low frequency, high magnitude) exercise on serum COMP and HA, and also investigated whether differences exist between males and females.

8.1 Summary of findings

This thesis demonstrates that femoral cartilage thickness can be measured reliably and accurately using US in healthy adults across a range of ages. This thesis also provided substantial normative data and revealed that baseline femoral cartilage thickness and serum biomarkers COMP, HA and lubricin have a large variability in healthy individuals across a

range of ages. Ageing was found to be negatively associated with cartilage thickness at the lateral femoral condyle. In contrast to the thesis hypotheses, ageing was not negatively associated with femoral cartilage thickness at either the notch or medial condyle, or with changes in serum COMP, HA, or lubricin. Overall, physical activity was unable to explain variation in these outcome measures and did not moderate the age-associated decrease in cartilage thickness at the lateral condyle. In addition, both cartilage thickness at all locations and serum COMP concentration were found to be lower in women compared to men. Moreover, acute exercise resulted in an increase in serum COMP and lubricin, which appeared independent of both exercise modality and sex. Unexpectedly, acute exercise did not result in an increase in serum HA, while the baseline concentrations were surprisingly higher in women compared to men.

8.2 Significance and future studies

The finding that femoral cartilage thickness can be measured reliably and accurately using US was particularly important given that previous research was limited. Moreover, this represent a significant finding for both clinicians and researchers, as the ability to assess femoral cartilage thickness provides an important investigative and potential diagnostic tool for conditions such as OA. In addition, although serum biomarkers offer a promising surrogate measure of articular cartilage and overall joint health, few studies have previously explored serum biomarkers in healthy individuals across a wide range of ages. Of particular significance was the normative data generated for serum HA and lubricin, which was previously very limited. Furthermore, by investigating how variables such as age, physical activity and anthropometric measures affect these markers, this thesis has provided valuable data for both clinicians and health professionals, which may in future be used to characterise what is ‘normal’ and thus what may be considered ‘abnormal’ serum biomarker concentrations.

A primary finding of this thesis was that age-associated changes in cartilage thickness and serum biomarkers are not a prerequisite of the ageing process. Overall, this finding was surprising, especially given that age is a major risk factor for knee OA (Anderson and Loeser, 2010). Importantly, this finding relates to this sample, who were healthy, of normal body weight, free from any recent knee injury and whom almost all engaged in some, or had a

history of regular physical activity. Previous research has indicated that ageing is associated with a reduction in knee cartilage morphology, including femoral cartilage thickness (Hudelmaier et al. 2001; Özçakar et al. 2014). The differences between the findings of the present study and previous research suggests that age-associated changes may be more prevalent in certain cartilage plates and/or moderated by other risk factors such as injury or body weight. Whether age is associated with changes to other cartilage plates, or to functional changes to the articular cartilage that were not tested in the present research requires further investigation.

This thesis also found that physical activity was not associated with any adverse change in cartilage thickness or serum biomarkers in healthy individuals. In addition, physical activity over the last 12 months demonstrated a potential positive relationship with cartilage thickness. Therefore, this thesis provides significant evidence that physical activity in healthy, normal weight individuals does not harm the knee joint. This supports previous studies indicating that physical activity does not increase the risk of OA (Bosomworth, 2009; Urquhart et al. 2011) and should help alleviate the potential concerns of both clinicians and the general public that exercise is detrimental for the knee joint. However, research is still required to determine whether extreme physical activity, or inactivity, has an adverse effect on the knee joint. In relation to the risk of OA, it could be that elite athletes, whom are accustomed to persistent high joint loading and have high injury rates are more likely to be associated with a greater risk of knee OA (Driban et al. 2015). Moreover, studies that have investigated the effect of bed rest on articular cartilage have previously been associated with both a reduction in cartilage thickness and a reduction in serum COMP concentrations (Liphardt et al. 2009), indicating that inactivity could be detrimental for the joint. Future studies should focus on the role on extreme activity and inactivity on cartilage biomarkers, with an aim being to determine whether a maximum or minimum loading volume exists to maintain joint health.

Additional factors, including, injury, sex, and anthropometric measures may also explain variations in these knee joint markers and were explored within this thesis. Research has previously demonstrated that knee injuries are associated with increased risk of future cartilage loss and OA. Several studies demonstrate that traumatic acute knee injuries such as cruciate and meniscal injuries are associated with increased serum COMP concentrations (Kuhne et al. 2014; Palmieri-Smith et al. 2016), which can remain elevated in prolonged

periods (Kuhne et al. 2014). However, several long term follow ups have indicated that both synovial joint COMP concentrations and serum concentrations fall within normal healthy values following extended periods of recovery (Åhlén et al. 2015; Cattano et al. 2016). This is supported by exploratory research in the present thesis (Appendix F), which demonstrated no difference in the joint markers of a small subset of participants who had reported a knee injury in the past. However, whether the elevated biomarker and inflammatory concentrations previously observed immediately post injury reflect a joint that is exposed to potential joint damage is unclear. Moreover, in agreement with the present study, femoral cartilage thicknesses (Faber et al. 2001; Özçakar et al. 2014) and baseline serum COMP concentrations have been shown to be lower in females compared to men (Jordan et al. 2003). These differences may explain why females have increased risk of knee OA compared to men and requires further investigation. Knee joint loading is also influenced by a person's physical characteristics. Body weight, body height and BMI all play a role in knee joint loading, and consequently may impact upon knee articular cartilage morphology and explain differences between males and females. In the present thesis, men with higher body weight and BMI were both associated with lower cartilage thickness at the lateral condyle, while in contrast, increased body weight was associated with thicker intercondyle notch cartilage. However, overall, as demonstrated in this thesis and elsewhere (Ding et al. 2005), the relationship between anthropometric measurements and joint markers are inconsistent.

In addition to baseline measures, the effect of acute loading on cartilage thickness and serum biomarkers has also been proposed as an additional method to assess the joint. Several previous studies have demonstrated how acute joint loading can result in an increase in serum COMP. Furthermore, increases in serum COMP have been associated changes in cartilage volume (Niehoff et al. 2011) and have predicted future cartilage loss (Erhart-Hledik et al. 2012). Crucially, prior to this thesis, few studies had previously explored the effect of acute exercise on changes in serum HA, lubricin or femoral cartilage thickness assessed by US. Therefore, this thesis provided an important opportunity to investigate the normal response of different acute loading modalities in healthy males and females. In relation to the acute response, this thesis was the first to demonstrate that serum lubricin increases in response to acute exercise. Furthermore, this thesis also demonstrated that in contrast to serum COMP, serum HA remains unchanged following acute loading. The difference in the response between HA and COMP was unexpected and suggests that both serum COMP and HA reflect different joint structures. Serum HA has been proposed as a marker of both cartilage

degradation and synovial inflammation, therefore, this finding may indicate that acute exercise does not result in any ill-effects on the joint. Furthermore, the fact that serum concentrations remained unchanged suggests that HA may remain within the joint despite an increase in joint load and intra-articular pressure in response to exercise. Moreover, given that HA is used as a therapeutic intervention for OA (Shimizu et al. 2010) and considered an important joint lubricant (Schmidt et al. 2007) this may be a positive finding.

Previous studies exploring different types of acute exercise on serum biomarkers are scarce. The present thesis extends this area of research and provides evidence that largely supports previous studies (Niehoff et al. 2011, Niehoff et al. 2010) suggesting that minimal differences exist in the serum biomarker response to different exercise modalities. In normal weight, healthy individuals, this research also provides evidence to indicate that weight bearing exercise does not impact cartilage any differently than non-weight bearing exercise. This is a significant finding given that weight bearing exercise is often considered to have a greater, and a potentially adverse effect on knee joint compared to non-weight bearing exercise. Although no significant differences were observed between weight bearing and non-weight bearing exercise in the present thesis, a recent study has demonstrated that the use of a weight vest (+40% body weight) independently increased the serum COMP response to walking compared to normal walking (Denning et al. 2015). Therefore, a person's body weight or size, rather the nature of the loading, may be important in the exercise response of serum biomarker. This is an area of research that warrants further research. Moreover, another noteworthy finding of the present thesis was that well trained runners completing a vigorous bout of acute running demonstrated the smallest acute increase in serum COMP. This suggests that training status and the type of exercise training may alter the exercise response of COMP to loading. The concept of functional adaptation of cartilage is supported by previous studies that have demonstrated that glycosaminoglycan content increases (Van Ginckel et al. 2010) and the exercise response of serum COMP to walking decreased following running training in previously sedentary runners (Celik et al. 2013). Importantly, the role of exercise training in the functional adaptation of cartilage tissue warrants further investigation.

In contrast to increases in serum COMP and lubricin, acute exercise did not result in a significant change in femoral cartilage thickness following either vigorous running or cycling exercise. Previous studies using MRI have typically demonstrated a decrease following

exercise (Eckstein et al. 2005). Despite US demonstrating strong reliability and measurement precision, differences may relate to the improved sensitivity of MRI compared to US and the ability to assess the thickness across the full femoral plate. In contrast to the present study, acute changes in femoral cartilage thickness assessed by US has been shown following walking and running (Harkey et al. 2017). Differences between their study and the present thesis may relate to differences in the fact that trained runners were investigated in the present thesis. Alternatively, differences may reflect how in the present study priority was given to venous sampling immediately post exercise and that US images were not obtained until approximately 5 minutes following cessation of the activity.

Overall, while cartilage thickness measurements and serum biomarkers are promising tools to monitor the knee joint, several limitations must be considered. Firstly, in relation to serum biomarkers, a recent review on biomarkers in OA acknowledged that currently no perfect markers to assess the knee joint currently exist (Lotz et al. 2013). COMP, HA and lubricin are also all expressed in several different tissues and are not exclusive to the joint. For example, serum COMP, which was initially proposed as a cartilage-specific molecule, has subsequently been shown to be synthesised by ligament and synovial fibroblasts (Müller et al. 1998) and therefore lacks tissue specificity. To date, serum COMP continues to be highly researched and has the most compelling evidence supporting its use as a marker of OA. As a marker of acute exercise, increases in COMP have been correlated with cartilage deformation (Niehoff et al. 2011) and with cartilage loss (Erhart-Hledik et al. 2012). More recently, in response to exercise, a decrease in COMP in the synovial fluid has been correlated with the increase in serum concentration (Hyldahl et al. 2016). However, unlike serum COMP, there is limited research regarding the relationship between lubricin and HA concentrations in the serum and at joint level. Further research is required, particularly in relation to HA and lubricin, to determine whether serum concentrations correspond with joint fluid concentrations and whether differences in serum concentrations at rest and in response to exercise reflect structural or functional changes at the joint. The development of assay techniques, particularly in relation to serum lubricin (as discussed in Chapter 6) may improve the ability to strengthen conclusions. Moreover, although the cartilage biomarkers in the present thesis were chosen based on their strengths compared to other markers, alternative markers continue to emerge and may also assist in the understanding of this area. Synovial fluid markers would also offer the ability to strengthen conclusions from the present studies.

However, studies using healthy individuals are often limited to small sample sizes due to the difficulties associated with obtaining synovial fluids from healthy joint tissue.

8.3 Conclusions

This thesis has advanced the understanding of several novel articular cartilage markers, including serum biomarkers and sonographic measurement of cartilage thickness by providing substantial normative data. Furthermore, using these cartilage biomarkers this thesis has provided important evidence regarding the effects of ageing and exercise on articular cartilage in healthy adults. Specifically, this research has provided evidence to suggest that ageing does not inevitably result in degenerative joint changes in healthy individuals (Chapter 5). This research also provides further evidence to indicate that higher levels of physical activity are not associated with adverse changes to the articular cartilage (Chapter 5). Therefore, while acute exercise may significantly increase cartilage turnover and joint lubrication, the effect appears to be temporary and appears well tolerated (Chapter 6). Importantly, the effect of acute exercise is largely independent of the type of exercise (Chapter 6 and 7), or the sex of the individual (Chapter 7). The findings from this thesis are significant given that femoral cartilage thickness and serum COMP, HA and lubricin have previously been associated with early degenerative change. Moreover, this research is important for the understanding of the pathophysiology of joint disease such as OA, and particularly, the role of ageing and physical activity. Overall, in healthy, normal weight individuals, both ageing and regular physical activity do not appear to lead toward joint degeneration. Future studies are particularly warranted in relation to extreme physical activity or inactivity, obesity and injury. Moreover, although serum biomarkers offer a promising tool to investigate the joint, more research is required to develop the relationship between serum concentrations and changes at the joint level.

9 References

Abraham, A. M., Goff, I., Pearce, M. S., Francis, R. M., Birrell, F., Fraser, B., Pearce, M. S., Francis, R. M., & Birrell, F. (2011). Reliability and validity of ultrasound imaging of features of knee osteoarthritis in the community. *BMC Musculoskeletal Disorders*, 12, 70.

Abraham, A. M., Pearce, M. S., Mann, K. D., Francis, R. M., & Birrell, F. (2014). Population prevalence of ultrasound features of osteoarthritis in the hand, knee and hip at age 63 years: The Newcastle thousand families birth cohort. *BMC Musculoskeletal Disorders*, 15, 162.

Abramson, S., & Krasnokutsky, S. (2006). Biomarkers in osteoarthritis. *Bulletin of the Hospital for Joint Diseases*. 64, 77–81.

Abusara, Z., Krawetz, R., Steele, B., DuVall, M., Schmidt, T., & Herzog, W. (2013). Muscular loading of joints triggers cellular secretion of PRG4 into the joint fluid. *Journal of Biomechanics*, 46, 1225–1230.

ACSM. (2010). Exercise prescription for other clinical populations: osteoporosis. In*:* W.R. Thompson, N.F. Gordon, S. Pescatello (Eds.), *ACSM's Guidelines for Exercise Testing and Prescription*. (pp.256 – 268). Balimore, MA: Lippincott, Williams and Wilkins.

Ahlen, M., Roshani, L., Liden, M., Struglics, A., Rostgard-Christensen, L., & Kartus, J. (2015). Inflammatory cytokines and biomarkers of cartilage metabolism 8 years after anterior cruciate ligament reconstruction: results from operated and contralateral knees. *American Journal of Sports Medicine*, 43, 1460–1466.

Akkaya, N., Akkaya, S., Ozcakar, L., Demirkan, F., Kiter, E., Konukcu, S., & Ardic, F. (2013a). Ultrasonographic measurement of the distal femoral cartilage thickness in patients with unilateral transtibial amputation. *Prosthetics and Orthotics International*, 37, 268–274.

Akkaya, S., Akkaya, N., Ozcakar, L., Kiliç, A., Sahin, F., Atalay, N. S., … Ardic, F., (2013b). Ultrasonographic evaluation of the femoral cartilage thickness after unilateral arthroscopic partial meniscectomy. *Knee Surgery, Sports Traumatology, Arthroscopy*, 21, 1104–1110.

Alford, J.W. (2005). Cartilage Restoration, Part 1: Basic Science, Historical Perspective, Patient Evaluation, and Treatment Options. *American Journal of Sports Medicine*, 33, 295–306.

Amin, S., LaValley, M. P., Guermazi, A., Grigoryan, M., Hunter, D. J., Clancy, M., … Felson, D. T. (2005). The relationship between cartilage loss on magnetic resonance imaging and radiographic progression in men and women with knee osteoarthritis. *Arthritis & Rheumatology*, 52, 3152–3159.

Anderson, S. A., & Loeser, R. F. (2010). Why is osteoarthritis an age-related disease? *Best Practice & Research: Clinical Rheumatology*, 24, 15–26.

Andersson, M. L. E., Petersson, I. F., Karlsson, K. E., Jonsson, E. N., Mansson, B., … Saxne, T. (2006a). Diurnal variation in serum levels of cartilage oligomeric matrix protein in patients with knee osteoarthritis or rheumatoid arthritis. *Annals of the Rheumatic Diseases*, 65, 1490–1494.

Andersson, M. L. E., Thorstensson, C. A., Roos, E. M., Petersson, I. F., Heinegård, D., & Saxne, T. (2006b). Serum levels of cartilage oligomeric matrix protein (COMP) increase temporarily after physical exercise in patients with knee osteoarthritis. *BMC Musculoskeletal Disorders*, 7, 98.

Andriacchi, T. P. (2013). Valgus alignment and lateral compartment knee osteoarthritis: A biomechanical paradox or new insight into knee osteoarthritis? *Arthritis & Rheumatology*, 65, 310–313.

Arendt, E., & Dick, R. (1995). Knee Injury Patterns Among Men and Women in Collegiate Basketball and Soccer: NCAA Data and Review of Literature. *American Journal of Sports Medicine*, 23, 694–701.

Arokoski, J. P. A., Jurvelin, J. S., Väätäinen, U., & Helminen, H. J. (2000). Normal and pathological adaptations of articular cartilage. *Scandinavian Journal of Medicine & Science in Sports*, 10, 186–198.

Arthritis Research UK. (2013). Osteoarthritis in general practice - Data and Perspectives - Arthritis Research UK. *The Medical Press*, 222, 253–258.

Astrand, P. O., & Rodahl, K. (1977). *Textbook of work physiology*. McGraw-Hill Book Company, New York.

Atkinson, A. J., Colburn, W. A., DeGruttola, V. G., DeMets, D. L., Downing, G. J., Hoth, D. F., … Zeger, S. L. (2001). Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. *Clinical Pharmacology & Therapeutics*, 69, 89-95.

Atkinson, G., & Nevill, A., (1998). Statistical methods for assessing measurement error (reliability) in variables relevant to sports medicine. *Sports Medicine*, 26, 217–238.

Attur, M., Krasnokutsky, S. S., Samuels, J., & Abramson, S. B. (2013). Prognostic biomarkers in osteoarthritis. *Current Opinion in Rheumatology*, 25, 136–144.

Baecke, J. A., Burema, J., & Frijters, J. E. (1982). A short questionanaire for the measuremnet of habitual physical activity in epidemiological studies. *The American Journal of Clinical Nutrition*, 36, 936–942.

Bauer, D. C., Hunter, D. J., Abramson, S. B., Attur, M., Corr, M., Felson, D., … Kraus, V. B. (2006). Classification of osteoarthritis biomarkers: a proposed approach. *Osteoarthritis and Cartilage*, 14, 723–727.

Bay-Jensen, A-C., Liu, Q., Byrjalsen, I., Li, Y., Wang, J., Pedersen, C., … Karsdal, M. A. (2011). Enzyme-linked immunosorbent assay (ELISAs) for metalloproteinase derived type II collagen neoepitope, CIIM-increased serum CIIM in subjects with severe radiographic osteoarthritis. *Clinical Biochemistry*, 44, 423–429.

Beckerman, H., Roebroeck, M. E., Lankhorst, G. J., Becher, J. G., Bezemer, P. D., & Verbeek, A. L. M. (2001). Smallest real difference, a link between reproducibility and responsiveness. *Quality of Life Research*, 10, 571–578.

Beckwée, D., Vaes, P., Cnudde, M., Swinnen, E., & Bautmans, I. (2013). Osteoarthritis of the knee: why does exercise work? A qualitative study of the literature. *Ageing Research Reviews*, 12, 226–236.

Bedson, J., & Croft, P.R. (2008). The discordance between clinical and radiographic knee osteoarthritis: a systematic search and summary of the literature. *BMC Musculoskeletal Disorders*, 9, 1–11.

Behringer, M., Montag, J., Kilian, Y., McCourt, M., Liphardt, A. M., & Mester, J. (2014). Serum cartilage oligomeric matrix protein: is there a repeated bout effect? *Orthopedic Reviews*, 6, 118–122.

Bemben, D. A., & Bemben, M. G., (2011). Dose-response effect of 40 weeks of resistance training on bone mineral density in older adults. *Osteoporosis International*, 22, 179–186.

Ben-Hur, H., Thole, H. H., Mashiah, A., Insler, V., Berman, V., Shezen, E., … Ornoy, A. (1997). Estrogen, progesterone and testosterone receptors in human fetal cartilaginous tissue: immunohistochemical studies. *Calcified Tissue International*, 60, 520–560.

Berenbaum, F. (2013). Osteoarthritis as an inflammatory disease (osteoarthritis is not osteoarthrosis!). *Osteoarthritis and Cartilage*, 21, 16–21.

Berry, P. A., Maciewicz, R. A., Wluka, A. E., Downey-Jones, M. D., Forbes, A., Hellawell, C. J., & Cicuttini, F. M. (2010). Relationship of serum markers of cartilage metabolism to imaging and clinical outcome measures of knee joint structure. *Annals of the Rheumatic Diseases*, 69, 1816–1822.

Blazek, K., Favre, J., Asay, J., Erhart-Hledik, J., & Andriacchi, T. (2014). Age and obesity alter the relationship between femoral articular cartilage thickness and ambulatory loads in individuals without osteoarthritis. *Journal of Orthopaedic Research*, 32, 394–402.

Bollen, S. (2000). Epidemiology of knee injuries: diagnosis and triage. *British Journal of Sports Medicine*, 34, 227–228.

Boocock, M., McNair, P., Cicuttini, F., Stuart, A., & Sinclair, T. (2009). The short-term effects of running on the deformation of knee articular cartilage and its relationship to biomechanical loads at the knee. *Osteoarthritis and Cartilage*,17, 883–890.

Borg, G. A. V. (1982). Psychophysical bases of perceived exertion. *Medicine & Science in Sports & Exercise*, 14, 377–381.

Bosomworth, N. J. (2009). Exercise and knee osteoarthritis: benefit or hazard? *Canadian Family Physician*, 55, 871–878.

Boyan, B. D., Hart, D. A, Enoka, R. M., Nicolella, D. P., Resnick, E., Berkley, K. J., … Kohrt, W. M. (2013). Hormonal modulation of connective tissue homeostasis and sex differences in risk for osteoarthritis of the knee. *Biology of Sex Differences*, 4, 3.

Brzycki, M. (1993). Strength Testing - Predicting a One-Rep Max from Reps-to-Fatigue. *Journal of Physical Education, Recreation and Dance*, 64, 88–90.

Buckwalter, J. A., & Mankin, H. J. (1997). Instructional course lectures, the American academy of orthopaedic surgeons - articular cartilage. Part I: tissue design and chondrocyte-matrix interactions. *Journal of Bone and Joint Surgery*, 79, 600–611.

Buckwalter, J., Mow, V., & Ratcliffe, A. (1994). Restoration of Injured or Degenerated Articular Cartilage. *The Journal of the American Academy of Orthopaedic Surgeons*, 2, 192–201.

Burstein, D., Bashir, A., & Gray, M. L. (2000). MRI techniques in early stages of cartilage disease. *Investigative Radiology*, 35, 622–638.

Cattano, N. M., Driban, J. B., Barbe, M. F., Tierney, R. T., Amin, M., & Sitler, M. R. (2016). Biochemical response to a moderate running bout in participants with or without a history of acute knee injury. *Journal of Athletic Training*, 51, 000–000.

Catterall, J. B., Thomas, V., Flannery, C. R. S., Kraus, V. B., & Stabler, T. (2010). Changes in serum and synovial fluid biomarkers after acute injury (NCT00332254). *Arthritis Research & Therapy*, 12, R229.

Celik, O., Salci, Y., Ak, E., Kalaci, A., & Korkusuz, F. (2013). Serum cartilage oligomeric matrix protein accumulation decreases significantly after 12 weeks of running but not swimming and cycling training - a randomised controlled trial. *Knee*, 20, 19–25.

Chakravarty, E. F., Hubert, H. B., Lingala, V. B., Zatarain, E., & Fries, J. F. (2008). Long distance running and knee osteoarthritis: a prospective study. *American Journal of Preventive Medicine*, 35, 133–138.

Chua, S. D. J., Messier, S. P., Legault, C., Lenz, M. E., Thonar, E. J. M. A., & Loeser, R. F. (2008). Effect of an exercise and dietary intervention on serum biomarkers in overweight and obese adults with osteoarthritis of the knee. *Osteoarthritis and Cartilage*, 16, 1047–1053.

Cibere, J., Zhang, H., Garnero, P., Poole, A. R., Lobanok, T., Saxne, T., … Esdaile, J. M., (2009). Association of biomarkers with pre-radiographically defined and radiographically defined knee osteoarthritis in a population-based study. *Arthritis & Rheumatology*, 60, 1372–1380.

Cicuttini, F., Forbes, A., Morris, K., Darling, S., Bailey, M., & Stuckey, S. (1999). Gender differences in knee cartilage volume as measured by magnetic resonance imaging. *Osteoarthritis and Cartilage*, 7, 265–271.

Clark, A. G., Jordan, J. M., Vilim, V., Renner, J. B., Dragomir, A. D., Luta, G., & Kraus, V. B. (1999). Serum cartilage oligomeric matrix protein reflects osteoarthritis presence and severity: The Johnston county osteoarthritis project. *Arthritis & Rheumatology*. 42, 2356–2364.

Clark, J. M. (1990). The structure of vascular channels in the subchondral plate. *Journal of Anatomy*, 171, 105–115.

Clarke, I. C. (1971). Articular cartilage: a review and scanning electron microscope study. 1. The interterritorial fibrillar architecture. *The Journal of Bone and Joint Surgery. British Volume*, 53, 732–750.

Conaghan, P. G., Hunter, D. J., Maillefert, J. F., Reichmann, W. M., Losina, E., Aliabadi, P., … Losina, E., (2011). Summary and recommendations of the OARSI FDA osteoarthritis assessment of structural change working group. *Osteoarthritis and Cartilage*, 19, 606–610.

Cotofana, S., Ring-Dimitriou, S., Hudelmaier, M., Himmer, M., Wirth, W., Sänger, A. M., & Eckstein, F. (2010). Effects of exercise intervention on knee morphology in middle-aged women: a longitudinal analysis using magnetic resonance imaging. *Cells Tissues Organs*, 192, 64–72.

Craig, C. L., Marshall, A. L., Sjöström, M., Bauman, A. E., Booth, M. L., Ainsworth, B. E., … Oja, P. (2003). International physical activity questionnaire: 12-Country reliability and validity. *Medicine & Science in Sports & Exercise*, 35, 1381–1395.

Criscione, L. G., Elliott, A. L., Stabler, T., Jordan, J. M., Pieper, C. F., & Kraus, V. B. (2005). Variation of serum hyaluronan with activity in individuals with knee osteoarthritis. *Osteoarthritis and Cartilage*. 13, 837–840.

Cymet, T. C., & Sinkov, V. (2006). Does long-distance running cause osteoarthritis? *The Journal of the American Osteopathic Association*, 106, 342–345.

D'Lima, D. D., Steklov, N., Patil, S., & Colwell, C. W. (2008). The Mark Coventry award: In vivo knee forces during recreation and exercise after knee arthroplasty. *Clinical Orthopaedics and Related Research*, 466, 2605–2611.

Dam, E. B., Byrjalsen, I., Karsdal, M. A., Qvist, P., & Christiansen, C. (2009). Increased urinary excretion of C-telopeptides of type II collagen (CTX-II) predicts cartilage loss over 21 months by MRI. *Osteoarthritis and Cartilage*, 17, 384–389.

Dargel, J., Feiser, J., Gotter, M., Pennig, D., & Koebke, J. (2009). Side differences in the anatomy of human knee joints. *Knee Surgery, Sports Traumatology, Arthroscopy*, 17, 1368–1376.

Denning, W. M., Pardo, M. B., Winward, J. G., Hunter, I., Ridge, S., Hopkins, J.T., … Seeley, M. K. (2016). Ambulation speed and corresponding mechanics are associated with changes in serum cartilage oligomeric matrix protein. *Gait Posture*, 44, 131–136.

Denning, W. M., Winward, J. G., Pardo, M. B., Hopkins, J. T., & Matthew, K. (2015). Body weight independently affects articular cartilage catabolism. *Journal of Sports Science & Medicine*, 14, 290–296.

Denning, W. M., Woodland, S., Winward, J. G., Leavitt, M. G., Parcell, A. C., Hopkins, J. T., … Seeley, M. K. (2014). The influence of experimental anterior knee pain during running on electromyography and articular cartilage metabolism. *Osteoarthritis and Cartilage*, 22, 1111–1119.

Ding, C., Cicuttini, F., Scott, F., Cooley, H., & Jones, G. (2005). Association between age and knee structural change: a cross sectional MRI based study. *Annals of the Rheumatic Diseases*, 64, 549–555.

Ding, C., Cicuttini, F., Scott, F., Glisson, M., & Jones, G. (2003). Sex differences in knee cartilage volume in adults: role of body and bone size, age and physical activity. *Rheumatology (Oxford)*, 42, 1317–1323.

Driban, J. B., Hootman, J. M., Sitler, M. R., Harris, K., & Cattano, N. M. (2015). Is participation in certain sports associated with knee osteoarthritis? A systematic review. *Journal of Athletic Training*, 50, 000–000.

Ebbeling, C. B., Ward, A., Puleo, E. M., Widrick, J., & Rippe, J. M. (1991). Development of a single-stage submaximal treadmill walking test. *Medicine & Science in Sports & Exercise*, 23, 966–973.

Eckstein, F., Cicuttini, F., Raynauld, J. P., Waterton, J.C., & Peterfy, C. (2006). Magnetic resonance imaging (MRI) of articular cartilage in knee osteoarthritis (OA): morphological assessment. *Osteoarthritis and Cartilage*, 14, A46–A75.

Eckstein, F., Lemberger, B., Gratzke, C., Hudelmaier, M., Glaser, C., Englmeier, K. H., Reiser, M. (2005). In vivo cartilage deformation after different types of activity and its dependence on physical training status. *Annals of the Rheumatic Diseases*, 64, 291–295.

Eckstein, F., Maschek, S., Wirth, W., Hudelmaier, M., Hitzl, W., Wyman, B. T., … Group, O. I. (2009). One-year change of knee cartilage morphology in the first release of participants from the osteoarthritis initiative progression subcohort: association with sex, body mass index, symptoms and radiographic osteoarthritis status. *Annals of the Rheumatic Diseases*, 68, 674–679.

Eckstein, F., Müller, S., Faber, S. C., Englmeier, K. H., Reiser, M., & Putz, R. (2002). Side differences of knee joint cartilage volume, thickness, and surface area, and correlation with lower limb dominance - An MRI-based study. *Osteoarthritis and Cartilage*, 10, 914–921.

Eckstein, F., Reiser, M., Englmeier, K. H., & Putz, R. (2001). In vivo morphometry and functional analysis of human articular cartilage with quantitative magnetic resonance imaging from image to data, from data to theory. *Anatomy and Embryology*, 203, 147–173.

Eckstein, F., Tieschky, M., Faber, S., Englmeier, K. H., & Reiser, M. (1999). Functional analysis of articular cartilage deformation, recovery, and fluid flow following dynamic exercise in vivo. *Anatomy and Embryology*, 200, 419–424.

Eckstein, F., Tieschky, M., Faber, S. C., Haubner, M., Kolem, H., Englmeier, K. H., & Reiser, M. (1998). Effect of physical exercise on cartilage volume and thickness in vivo: MR imaging study. *Radiology*, 207, 243–248.

El-Arman, M. M., El-Fayoumi, G., El-Shal, E., El-Boghdady, I., & El-Ghaweet, A. (2010). Aggrecan and cartilage oligomeric matrix protein in serum and synovial fluid of

patients with knee osteoarthritis. *The Musculoskeletal Journal of Hospital for Special Surgery*, 6, 171–176.

Eliasziw, M., Young, S. L., Woodbury, M. G., & Fryday-Field, K. (1994). Statistical methodology for the concurrent assessment of interrater and intrarater reliability: using goniometric measurements as an example. *Physical Therapy*, 74, 777–788.

Elliott, A. L., Kraus, V. B., Luta, G., Stabler, T., Renner, J. B., Woodard, J., … Jordan, J. M. (2005). Serum hyaluronan levels and radiographic knee and hip osteoarthritis in African Americans and caucasians in the Johnston county osteoarthritis project. *Arthritis & Rheumatology*, 52, 105–111.

Elsaid, K. A., Zhang, L., Waller, K., Tofte, J., Teeple, E., Fleming, B. C., & Jay, G.D. (2012). The impact of forced joint exercise on lubricin biosynthesis from articular cartilage following ACL transection and intra-articular lubricin's effect in exercised joints following ACL transection. *Osteoarthritis and Cartilage*, 20, 940–948.

Englund, M., Guermazi, A., Roemer, F. W., Aliabadi, P., Yang, M., Lewis, C. E., … Felson, D. T. (2009). Meniscal tear in knees without surgery and the development of radiographic osteoarthritis among middle-aged and elderly persons: the multicenter osteoarthritis study. *Arthritis & Rheumatology*, 60, 831–839.

Engström-Laurent, A., & Hällgren, R. (1987). Circulating hyaluronic acid levels vary with physical activity in healthy subjects and in rheumatoid arthritis patients. *Arthritis & Rheumatology*, 30, 1333–1338.

Engström-Laurent, A., Laurent, U. B. G., Lilja, K., & Laurent, T. C. (1985). Concentration of sodium hyaluronate in serum. *Scandinavian Journal of Clinical and Laboratory Investigation*, 45, 497–504.

Erhart-Hledik, J. C., Favre, J., Asay, J. L., Smith, R. L., Giori, N. J., Mundermann, A., … Andriacchi, T. P. (2012). A relationship between mechanically-induced changes in serum cartilage oligomeric matrix protein (COMP) and changes in cartilage thickness after 5 years. *Osteoarthritis and Cartilage*, 20, 1309–1315.

Esser, S., & Bailey, A., (2011). Effects of exercise and physical activity on knee osteoarthritis. *Current Pain and Headache Reports*, 15, 423–430.

Faber, S. C., Eckstein, F., Lukasz, S., Mühlbauer, R., Hohe, J., Englmeier, K.H., & Reiser, M. (2001). Gender differences in knee joint cartilage thickness, volume and articular surface areas: assessment with quantitative three-dimensional MR imaging. *Skeletal Radiology*, 30, 144–150.

Felson, D. T., Hannan, M. T., Naimark, A., Berkeley, J., Gordon, G., Wilson, P. W. F., & Anderson, J. (1991). Occupational physical demands, knee bending, and knee osteoarthritis: results from the framingham study. *The Journal of Rheumatology*, 18, 1587–1592.

Felson, D. T., Naimark, A., Anderson, J., Kazis, L., Castelli, W., & Meenan, R. F. (1987). The prevalence of knee osteoarthritis in the elderly. The framingham osteoarthritis study. *Arthritis & Rheumatology*, 30, 914–918.

Felson, D.T., Zhang, Y., Hannan, M. T., Naimark, A., Weissman, B., & Aliabadi, P. (1995). The incidence and natural history of knee osteoarthritis in the elderly. The Framingham Osteoarthritis Study. *Arthritis & Rheumatology*, 38 (10), 1500-1505.

Felson, D.T., Zhang, Y., Hannan, M. T., Naimark, A., Weissman, B., Aliabadi, P., & Levy, D. (1997). Risk factors for incident radiographic knee osteoarthritis in the elderly: the Framingham Study. *Arthritis & Rheumatology*, 40 (4), 728-733.

Foley, S., Ding, C., Cicuttini, F., & Jones, G. (2007). Physical activity and knee structural change: a longitudinal study using MRI. *Medicine & Science in Sports & Exercise*, 39, 426–434.

Forsblad d'Elia, H., Christgau, S., Mattsson, L-A., Saxne, T., Ohlsson, C., Nordborg, E., & Carlsten, H. (2004). Hormone replacement therapy, calcium and vitamin D3 versus calcium and vitamin D3 alone decreases markers of cartilage and bone metabolism in rheumatoid arthritis: a randomized controlled trial [ISRCTN46523456]. *Arthritis Research & Therapy*, 6, R457-68.

Fox, A. J. S., Bedi, A., & Rodeo, S. A. (2009). The basic science of articular cartilage: structure, composition, and function. *Sports Health*, 1, 461–468.

Franciozi, C. E. S., Tarini, V. A. F., Reginato, R. D., Gonçalves, P. R. S., Medeiros, V. P., Ferretti, M., … Faloppa, F. (2013). Gradual strenuous running regimen predisposes to osteoarthritis due to cartilage cell death and altered levels of glycosaminoglycans. *Osteoarthritis and Cartilage*, 21, 965–972.

Gage, B. E., McIlvain, N. M., Collins, C. L., Fields, S. K., & Comstock, R. D. (2012). Epidemiology of 6.6 million knee injuries presenting to United States emergency departments from 1999 through 2008. *Academic Emergency Medicine*, 19 (4), 378–385.

Galois, L., Etienne, S., Grossin, L., Watrin-Pinzano, A., Cournil-Henrionnet, C., Loeuille, D., … Gillet, P. (2004). Dose-response relationship for exercise on severity of

experimental osteoarthritis in rats: a pilot study. *Osteoarthritis and Cartilage*, 12, 779–786.

Garnero, P., Piperno, M., Gineyts, E., Christgau, S., Delmas, P. D., & Vignon, E. (2001). Cross sectional evaluation of biochemical markers of bone, cartilage, and synovial tissue metabolism in patients with knee osteoarthritis: relations with disease activity and joint damage. *Annals of the Rheumatic Diseases*, 60, 619–626.

Garnero, P., Rousseau, J. C., & Delmas, P. D. (2000). Molecular basis and clinical use of biochemical markers of bone, cartilage, and synovium in joint diseases. *Arthritis & Rheumatology*, 43, 953–968.

Germaschewski, F. M., Matheny, C. J., Larkin, J., Liu, F., Thomas, L. R., Saunders, J. S., … Graham, N. M. (2014). Quantitation of ARGS aggrecan fragments in synovial fluid, serum and urine from osteoarthritis patients. *Osteoarthritis and Cartilage*, 22, 690–697.

Goldring, M. B. (2012). Chondrogenesis, chondrocyte differentiation, and articular cartilage metabolism in health and osteoarthritis. *Therapeutic Advances in Musculoskeletal Disease*, 4, 269–285.

Golightly, Y. M., Marshall, S. W., Kraus, V. B., Renner, J. B., Villaveces, A., Casteel, C., & Jordan, J. M. (2011). Biomarkers of incident radiographic knee osteoarthritis: do they vary by chronic knee symptoms? *Arthritis & Rheumatology*, 63, 2276–2283.

Gratzke, C., Hudelmaier, M., Hitzl, W., Glaser, C., & Eckstein, F. (2007). Knee cartilage morphologic characteristics and muscle status of professional weight lifters and sprinters: a magnetic resonance imaging study. *American Journal of Sports Medicine*, 35, 1346–1353.

Grotle, M., Hagen, K. B., Natvig, B., Dahl, F. A., & Kvien, T. K. (2008). Obesity and osteoarthritis in knee, hip and/or hand: an epidemiological study in the general population with 10 years follow-up. *BMC Musculoskeletal Disorders*, 9, 132.

Guilak, F. (2011). Biomechanical factors in osteoarthritis. *Best Practice & Research: Clinical Rheumatology*, 25, 815–23.

Hanna, F., Ebeling, P. R., Wang, Y., O'Sullivan, R., Davis, S., Wluka, A. E., & Cicuttini, F. M. (2005). Factors influencing longitudinal change in knee cartilage volume measured from magnetic resonance imaging in healthy men. *Annals of the Rheumatic Diseases*, 64, 1038–42.

Hannan, M. T., Felson, D. T., Anderson, J. J., & Naimark, A. (1993). Habitual physical activity is not associated with knee osteoarthritis: The Framingham Study. *The Journal of Rheumatology*, 20, 704–709.

Harkey, M. S., Blackburn, J. T., Davis, H., Sierra-Arevalo, L., Nissman, D., & Pietrosimone, B. (2017). Ultrasonographic assessment of medial femoral cartilage deformation acutely following walking and running. *Osteoarthritis and Cartilage*, 25, 907–913.

Hart, D. J., Doyle, D.V., & Spector, T. D. (1999). Incidence and risk factors for radiographic knee osteoarthritis in middle-aged women: the Chingford Study. *Arthritis & Rheumatism*, 42, 17-24.

Hayes, A. F. (2012). PROCESS: A versatile computational tool for observed variable mediation, moderation, and conditional process modelling. http://www.afhayes.com/public/process2012.pdf

Helmark, I. C., Mikkelsen, U. R., Børglum, J., Rothe, A., Petersen, M.C.H., Andersen, O., Langberg, H., & Kjaer, M. (2010). Exercise increases interleukin-10 levels both intraarticularly and peri-synovially in patients with knee osteoarthritis: a randomized controlled trial. *Arthritis Research & Therapy*, 12, R126.

Helmark, I. C., Petersen, M. C. H., Christensen, H. E., Kjaer, M., & Langberg, H. (2012). Moderate loading of the human osteoarthritic knee joint leads to lowering of intraarticular cartilage oligomeric matrix protein. *Rheumatology International*, 32, 1009–1014.

Heywood, V. (1998). The physical fitness specialist certification manual. In: Heywood, V. (Ed.), *Advanced Fitness Assessment & Exercise Prescription*. (pp. 48). Leeds: Human Kinetics.

Hinghofer-Szalkay, H. G., Mekonen, W., Rössler, A., Schwaberger, G., Lamprecht, M., & Hofmann, P. (2002). Post-exercise decrease of plasma hyaluronan: increased clearance or diminished production? *Physiological Research*, 51, 139–144.

Hinterwimmer, S., Feucht, M.J., Steinbrech, C., Graichen, H., & Von Eisenhart-Rothe, R. R, (2013). The effect of a six-month training program followed by a marathon run on knee joint cartilage volume and thickness in marathon beginners. *Knee Surgery, Sports Traumatology, Arthroscopy*, 22, 1353–1359.

Hoch, J. M., Mattacola, C. G., Bush, H. M., Medina McKeon, J. M., Hewett, T. E., & Lattermann, C. (2012). Longitudinal documentation of serum cartilage oligomeric matrix protein and patient reported outcomes in collegiate soccer athletes over the course of an athletic season. *American Journal of Sports Medicine*, 40, 2583–2589.

Hudelmaier, M., Glaser, C., Englmeier, K.-H., Reiser, M., Putz, R., Eckstein, F., (2003). Correlation of knee-joint cartilage morphology with muscle cross-sectional areas vs.

anthropometric variables. *The anatomical record. Part A, Discoveries in molecular, cellular, and evolutionary biology*. 270, 175–184.

Hudelmaier, M., Glaser, C., Hohe, J., Englmeier, K. H., Reiser, M., Putz, R., & Eckstein, F. (2001). Age-related changes in the morphology and deformational behavior of knee joint cartilage. *Arthritis & Rheumatology*, 44, 2556–2561.

Hunt, M. A., Pollock, C. L., Kraus, V. B., Saxne, T., Peters, S., Huebner, J. L., … Cibere, J. (2013). Relationships amongst osteoarthritis biomarkers, dynamic knee joint load, and exercise: results from a randomized controlled pilot study. *BMC Musculoskeletal Disorders* 14, 115.

Hunter, D. J., Arden, N., Conaghan, P. G., Eckstein, F., Gold, G., Grainger, A., …Zhang, W. (2011). Definition of osteoarthritis on MRI: results of a Delphi exercise. *Osteoarthritis and Cartilage*, 19, 963–969.

Hunter, D. J., Mcdougall, J. J., Keefe, F. J., England, N., Hospital, B., Ave, P. H., & Ma, B. (2009). The symptoms of OA and the genesis of pain. *Rheumatic Diseases Clinics of North America*, 34, 1–19.

Hunziker, E. (2002). Quantitative structural organization of normal adult human articular cartilage. *Osteoarthritis and Cartilage*, 10, 564–572.

Hyldahl, R. D., Evans, A., Kwon, S., Ridge, S. T., Robinson, E., Hopkins, J. T., & Seeley, M. K. (2016). Running decreases knee intra-articular cytokine and cartilage oligomeric matrix concentrations: a pilot study. *European Journal of Applied Physiology*, 116, 2305–2314.

Iagnocco, A. (2010). Imaging the joint in osteoarthritis: a place for ultrasound? *Best Practice & Research: Clinical Rheumatology*, 24, 27–38.

Inoue, R., Ishibashi, Y., Tsuda, E., Yamamoto, Y., Matsuzaka, M., Takahashi, I., … Toh, S. (2011). Knee osteoarthritis, knee joint pain and aging in relation to increasing serum hyaluronan level in the Japanese population. *Osteoarthritis and Cartilage*, 19, 51–57.

Ishijima, M., Watari, T., Naito, K., Kaneko, H., Futami, I., Yoshimura-Ishida, K., … Kaneko, K. (2011). Relationships between biomarkers of cartilage, bone, synovial metabolism and knee pain provide insights into the origins of pain in early knee osteoarthritis. *Arthritis Research & Therapy*, 13, R22.

Jay, G. D., Torres, J. R., Rhee, D. K., Helminen, H. J., Hytinnen, M. M., Cha, C-J., … Warman, M. L. (2007a). Association between friction and wear in diarthrodial joints lacking lubricin. *Arthritis & Rheumatology*, 56, 3662–3669.

Jay, G. D., Torres, J. R., Warman, M. L., Laderer, M. C., & Breuer, K.S. (2007b). The role of lubricin in the mechanical behavior of synovial fluid. *Proceedings of the National Academy of Sciences of the United States of America*, 104, 6194–6199.

Jones, A. R. C., & Flannery, C. R. (2007). Bioregulation of lubricin expression by growth factors and cytokines. *European Cells and Materials*, 13, 40–45.

Jones, G., Ding, C., Scott, F., Glisson, M., & Cicuttini, F. (2004). Early radiographic osteoarthritis is associated with substantial changes in cartilage volume and tibial bone surface area in both males and females. *Osteoarthritis and Cartilage*, 12, 169–174.

de Jong, Z., Munneke, M., Vilim, V., Zwinderman, A. H., Kroon, H.M., Ronday, H.K., … Degroot, J. (2008). Value of serum cartilage oligomeric matrix protein as a prognostic marker of large-joint damage in rheumatoid arthritis--data from the RAPIT study. *Rheumatology (Oxford)*, 47, 868–871.

Jordan, J. M., Luta, G., Stabler, T., Renner, J. B., Dragomir, A. D., Vilim, V., … Kraus, V. B. (2003). Ethnic and sex differences in serum levels of cartilage oligomeric matrix protein: The Johnston county osteoarthritis project. *Arthritis & Rheumatology*, 48, 675–681.

Kane, D., Balint, P. V., Sturrock, R., & Grassi, W. (2004). Musculoskeletal ultrasound-a state of the art review in rheumatology. Part 1: current controversies and issues in the development of musculoskeletal ultrasound in rheumatology. *Rheumatology (Oxford)*, 43, 823–828.

Kara, M., Tiftik, T. T. T., Öken, Ö., Akkaya, N., Tunc, H., Özçakar, L., … Ozcakar, L. (2013). Ultrasonographic measurement of femoral cartilage thickness in patients with spinal cord injury. *Journal of Rehabilitative Medicine*, 45, 145–148.

Karvonen, R. L., Negendank, W. G., Teitge, R. A., Reed, A. H., Miller, P. R., & Fernandez-Madrid, F. (1994). Factors affecting articular cartilage thickness in osteoarthritis and aging. *The Journal of Rheumatology*, 21, 1310–1318.

Kaya, A., Kara, M., Tiftik, T. T., Tezcan, M. E., Öztürk, M. A., Akıncı, A., …Ozcakar, L. (2012). Ultrasonographic evaluation of the femoral cartilage thickness in patients with systemic lupus erythematosus. *Rheumatology International*, 33, 899–901.

Kazam, J. K., Nazarian, L. N., Miller, T. T., Sofka, C. M., Parker, L., & Adler, R. S. (2011). Sonographic evaluation of femoral trochlear cartilage in patients with knee pain. *Journal of Ultrasound in Medicine*, 30, 797–802.

Kersting, U. G., Stubendorff, J. J., Schmidt, M. C., & Brüggemann, G-P. (2005). Changes in knee cartilage volume and serum COMP concentration after running exercise. *Osteoarthritis and Cartilage*, 13, 925–934.

Kessler, M. A., Glaser, C., Tittel, S., Reiser, M., & Imhoff, A. B. (2008). Recovery of the menisci and articular cartilage of runners after cessation of exercise: additional aspects of in vivo investigation based on 3-dimensional magnetic resonance imaging. *American Journal of Sports Medicine*, 36, 966–970.

Kessler, M. A., Glaser, C., Tittel, S., Reiser, M., & Imhoff, A. B. (2006). Volume changes in the menisci and articular cartilage of runners: an in vivo investigation based on 3-D magnetic resonance imaging. *American Journal of Sports Medicine*, 34, 832–836.

Kim, H. J., Lee, Y. H., & Kim, C. K. (2009). Changes in serum cartilage oligomeric matrix protein (COMP), plasma CPK and plasma hs-CRP in relation to running distance in a marathon (42.195 km) and an ultra-marathon (200 km) race. *European Journal of Applied Physiology*, 105, 765–770.

Kong, S. Y., Stabler, T. V, Criscione, L. G., Elliott, A. L., Jordan, J. M., & Kraus, V. B. (2006). Diurnal variation of serum and urine biomarkers in patients with radiographic knee osteoarthritis. *Arthritis & Rheumatology*, 54, 2496–2504.

Koo, S., Gold, G. E. E., & Andriacchi, T. P. P. (2005). Considerations in measuring cartilage thickness using MRI: factors influencing reproducibility and accuracy. *Osteoarthritis and Cartilage*, 13, 782–789.

Krasnokutsky, S., Attur, M., Palmer, G., Samuels, J., & Abramson, S. B. (2008). Current concepts in the pathogenesis of osteoarthritis. *Osteoarthritis and Cartilage*, 16, S1-3.

Kuhne, S. A., Neidhart, M., Everson, M. P., Hantzschel, H., Fine, P. R., Gay, S., … Gay, R. E. (2014). Persistent high serum levels of cartilage oligomeric matrix protein in a subgroup of patients with traumatic knee injury. *Rheumatology International*, 18, 21–25.

Kutzner, I., Heinlein, B., Graichen, F., Rohlmann, A., Halder, A. M., Beier, A., & Bergmann, G. (2012). Loading of the knee joint during ergometer cycling: telemetric in vivo data. *Journal of Orthopaedic & Sports Physical Therapy*, 42, 1032–1038.

Lafeber, F. P. J. G., & van Spil, W. E. (2013). Osteoarthritis year 2013 in review: Biomarkers; reflecting before moving forward, one step at a time. *Osteoarthritis and Cartilage*, 21, 1452–1464.

Larsson, S., Lohmander, L. S., & Struglics, A. (2009). Synovial fluid level of aggrecan ARGS fragments is a more sensitive marker of joint disease than glycosaminoglycan or aggrecan levels: a cross-sectional study. *Arthritis Research & Therapy*, 11, R92.

Lavie, C. J., Thomas, R. J., Squires, R. W., Allison, T. G., & Milani, R. V. (2009). Exercise training and cardiac rehabilitation in primary and secondary prevention of coronary heart disease. *Mayo Clinic Proceedings*, 84, 373–383.

Law, R-J., Saynor, Z. L., Gabbitas, J., Jones, J., Kraus, A., Breslin, A., … Thom, J. M. (2015). The effects of aerobic and resistance exercise on markers of large joint health in stable rheumatoid arthritis patients: a pilot study. *Musculoskeletal Care*, 13, 222–235.

Leong, D. J., & Sun, H. B. (2014). Mechanical Loading: Potential Preventative and Therapeutic Strategy for Osteoarthritis. *J Am Acad Orthop Surg*, 22, 465–466.

Levick, J. R., & McDonald, J. N. (1995). Fluid movement across synovium in healthy joints: role of synovial fluid macromolecules. *Ann. Rheum. Dis*, 54, 417–423.

Lindqvist, U., Engström-Laurent, A., Laurent, U., Nyberg, A., Björklund, U., Eriksson, H., … Tengblad, A. (2010). The diurnal variation of serum hyaluronan in health and disease. *Scand. J. Clin. Lab. Invest*, 48, 765-770.

Liphardt, A-M., Mundermann, A., Koo, S., Backer, N., Andriacchi, T. P., Zange, J., … Heer, M. (2009). Vibration training intervention to maintain cartilage thickness and serum concentrations of cartilage oligometric matrix protein (COMP) during immobilization. *Osteoarthr. Cartil*, 17, 1598–1603.

Liu, F., Wang, X., Zhang, X., Ren, C., & Xin, J. (2016). Role of Serum cartilage oligomeric matrix protein (COMP) in the diagnosis of rheumatoid arthritis (RA): A case–control study. *Journal of International Medical Research*, 44, 940–949.

Lohmander, L. S., Dahlberg, L., Ryd, L., & Heinegard, D. (1989). Increased levels of proteoglycan fragments in knee joint fluid after injury. *Arthritis & Rheumatology*, 32, 1434–1442.

Lohmander, L. S., Ionescu, M., Jugessur, H., & Poole, A. R. (1999). Changes in joint cartilage aggrecan after knee injury and in osteoarthritis. *Arthritis & Rheumatology*, 42, 534–544.

Lohmander, L. S., Roos, H., Dahlberg, L., Hoerrner, L. A., & Lark, M. W. (1994). Temporal patterns of stromelysin-1, tissue inhibitor, and proteoglycan fragments in human knee joint fluid after injury to the cruciate ligament or meniscus. *Journal of Orthopaedic Research*, 12, 21–28.

Lotz, M., Martel-Pelletier, J., Christiansen, C., Brandi, M-L., Bruyère, O., Chapurlat, R., … Reginster, J.-Y. (2013). Value of biomarkers in osteoarthritis: current status and perspectives. *Annals of the Rheumatic Diseases*, 72, 1756–1763.

Luria, A., & Chu, C. R. (2013). Articular cartilage changes in maturing athletes: new targets for joint rejuvenation. *Sports Health: A Multidisciplinary Approach*, 6, 18–30.

Malas, F. U., Kara, M., Kaymak, B., Akinci, A., & Özçakar, L. (2014). Ultrasonographic evaluation in symptomatic knee osteoarthritis: clinical and radiological correlation. *International Journal of Rheumatic Diseases*, 17, 536–540.

Malas, F. U., Kara, M., Aktekin, L., Ersöz, M., Özçakar, L., Ersoz, M., & Özçakar, L. (2013). Does vitamin D affect femoral cartilage thickness? An ultrasonographic study. *Clinical Rheumatology*, 33, 1–4.

Manicourt, D. H., Poilvache, P., Nzeusseu, A., Van Egeren, A., Devogelaer, J. P., Lenz, M. E., & Thonar, E. J. M. A., (1999). Serum levels of hyaluronan, antigenic keratan sulfate, matrix metalloproteinase 3, and tissue inhibitor of metalloproteinases 1 change predictably in rheumatoid arthritis patients who have begun activity after a night of bed rest. *Arthritis & Rheumatology*, 42, 1861–1869.

Mankin, H. J., Mow, V. C., Buckwalter, J. A., Iannotti, J. P., & Ratcliffe, A. (1999). Articular cartilage structure, composition, and function. In: Buckwalter, J. A., Einhorn, T. A., & Simon, S. R. (Eds.), *Orthopaedic Basic Science: Biology and Biomechanics of the Musculoskeletal System. American academy of orthopaedic surgeons* (pp. 444–470), Rosemount, IL.

Marcelino, J., Carpten, J. D., Suwairi, W. M., Gutierrez, O. M., Schwartz, S., Robbins, C., … Warman, M. L. (1999). CACP, encoding a secreted proteoglycan, is mutated in camptodactyly-arthropathy-coxa vara-pericarditis syndrome. *Nat. Genet*, 23, 319–322.

Maroudas, A. (1979). Physicochemical properties of articular cartilage. In: Freeman, M. (Ed.), *Adult Articular Cartilage* (pp. 215-290). Kent: Pitman Med.

Maroudas, A. (1976). Balance between swelling pressure and collagen tension in normal and degenerate cartilage. *Nature*, 260, 808–809.

Martin, J. A., & Buckwalter, J. A. (2002). Aging, articular cartilage chondrocyte senescence and osteoarthritis. *Biogerontology*, 3, 257–264.

Matzat, S. J., van Tiel, J., Gold, G. E., & Oei, E. H. G. (2013). Quantitative MRI techniques of cartilage composition. *Quantitative Imaging in Medicine Surgery*, 3, 162-I74.

Mosher, T. J., Liu, Y., & Torok, C.M. (2010). Functional cartilage MRI T2 mapping: evaluating the effect of age and training on knee cartilage response to running. *Osteoarthritis and Cartilage*, 18, 358–364.

Mow, V. C., & Guo, X. E. (2002). Mechano-electrochemical properties of articular cartilage: their inhomogeneities and anisotropies. *Annu. Rev. Biomed. Eng*, 4, 175–209.

Mow, V. C., Holmes, M. H., & Lai, W. M. (1984). Fluid transport and mechanical properties of articular cartilage: A review. *Journal of Biomechanics*, 17, 377-394.

Muhlbauer, R., Lukasz, T. S., Faber, T. S., Stammberger, T., & Eckstein, F. (2000). Comparison of knee joint cartilage thickness in triathletes and physically inactive volunteers based on magnetic resonance imaging and three-dimensional analysis. *American Journal of Sports Medicine*, 28, 541–546.

Muir, H., Bullough, P., & Maroudas, A. (1970). The distribution of collagen in human articular cartilage with some of its physiological implications. *The Journal of Bone and Joint Surgery. British Volume*, 52, 554–563.

Müller, G., Michel, A., & Altenburg, E. (1998). COMP (cartilage oligomeric matrix protein) is synthesized in ligament, tendon, meniscus, and articular cartilage. *Connective Tissue Research*, 39, 233–244.

Multanen, J., Rauvala, E., Lammentausta, E., Ojala, R., Kiviranta, I., Häkkinen, A., Nieminen, M. T., …Heinonen, A. (2009). Reproducibility of imaging human knee cartilage by delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) at 1.5 Tesla. *Osteoarthritis and Cartilage*, 17, 559–564.

Mündermann, A., Dyrby, C. O., Andriacchi, T. P., & King, K. B. (2005). Serum concentration of cartilage oligomeric matrix protein (COMP) is sensitive to physiological cyclic loading in healthy adults. *Osteoarthritis and Cartilage*, 13, 34–38.

Mündermann, A., King, K. B., Smith, R. L., & Andriacchi, T. P. (2009). Change in serum COMP concentration due to ambulatory load is not related to knee OA status. *Journal of Orthopaedic Research*, 27, 1408–1413.

Munro, B. (2005). Correlation. In B. Munro (Ed.), *Statistical methods for health care research*. (5th ed., pp. 239–258). Philadelphia: Lippincott, Williams and Wilkins.

Musumeci, G., Castrogiovanni, P., Trovato, F. M., Imbesi, R., Giunta, S., Szychlinska, M. A., … Mobasheri, A. (2014). Physical activity ameliorates cartilage degeneration in a rat model of aging: A study on lubricin expression. *Scandinavian Journal of Medicine & Science in Sports*, 25, 1–9.

Muthuri, S. G., McWilliams, D. F., Doherty, M., & Zhang, W. (2011). History of knee injuries and knee osteoarthritis: A meta-analysis of observational studies. *Osteoarthritis and Cartilage*, 19, 1286–1293.

Naredo, E., Acebes, C., Möller, I., Canillas, F., de Agustín, J. J., de Miguel, E., … Sáenz-Navarro, I. (2009). Ultrasound validity in the measurement of knee cartilage thickness. *Annals of the Rheumatic Diseases*. 68, 1322–1327.

Neidhart, M., Hauser, N., Paulsson, M., DiCesare, P. E., Michel, B. A., & Hauselmann, H. J., (1997). Small Fragments of Cartilage Oligomeric Matrix Protein in Synovial Fluid and Serum as Markers for Cartilage Degeneration. *Rheumatology*, 36, 1151–1160.

Neidhart, M., Müller-Ladner, U., Frey, W., Bosserhoff, A. K., Colombani, P. C., Frey-Rindova, P., … Gay, S. (2000). Increased serum levels of non-collagenous matrix proteins (cartilage oligomeric matrix protein and melanoma inhibitory activity) in marathon runners. *Osteoarthritis and Cartilage*, 8, 222–229.

Ni, G-X., Lei, L., & Zhou, Y-Z., (2012). Intensity-dependent effect of treadmill running on lubricin metabolism of rat articular cartilage. *Arthritis Research & Therapy*, 14, R256.

Ni, G-X., Zhan, L-Q., Gao, M-Q., Lei, L., Zhou, Y-Z., & Pan, Y-X. (2011). Matrix metalloproteinase-3 inhibitor retards treadmill running-induced cartilage degradation in rats. *Arthritis Research & Therapy*, 13, R192.

Niehoff, A., Kersting, U. G., Helling, S., Dargel, J., Maurer, J., Thevis, M., … Brüggemann, G-P. (2010). Different mechanical loading protocols influence serum cartilage oligomeric matrix protein levels in young healthy humans. *European Journal of Applied Physiology*, 110, 651–657.

Niehoff, A., Muller, M., Bruggemann, L., Savage, T., Zaucke, F., Eckstein, F., … Bruggemann, G-P. (2011). Deformational behaviour of knee cartilage and changes in serum cartilage oligomeric matrix protein (COMP) after running and drop landing. *Osteoarthritis and Cartilage*, 19, 1003–1010.

Nugent-Derfus, G. E., Takara, T., O'Neill, J. K., Cahill, S. B., Görtz, S., Pong, T., … Sah, R. L. (2007). Continuous passive motion applied to whole joints stimulates chondrocyte biosynthesis of PRG4. *Osteoarthritis and Cartilage*, 15, 566–574.

Nugent, G. E., Aneloski, N. M., Schmidt, T. A, Schumacher, B. L., Voegtline, M. S., & Sah, R. L. (2006). Dynamic shear stimulation of bovine cartilage biosynthesis of proteoglycan 4. *Arthritis & Rheumatology*, 54, 1888–1896.

O'Reilly, S. C., Muir, K. R., & Doherty, M. (1999). Effectiveness of home exercise on pain and disability from osteoarthritis of the knee: a randomised controlled trial. *Annals of the Rheumatic Diseases*, 9, 15–19.

Øiestad, B. E., Engebretsen, L., Storheim, K., & Risberg, M. A. (2009). Knee osteoarthritis after anterior cruciate ligament injury: a systematic review. *American Journal of Sports Medicine*, 37, 1434–1443.

Otterness, I. G., & Eckstein, F. (2007). Women have thinner cartilage and smaller joint surfaces than men after adjustment for body height and weight. *Osteoarthritis and Cartilage*, 15, 666–672.

Özçakar, L., Tunç, H., Öken, Ö., Ünlü, Z., Durmu, B., Ozcakar, L., … Ozgocmen, S. (2014). Femoral cartilage thickness measurements in healthy individuals: learning, practicing and publishing with TURK-MUSCULUS. *Journal of Back and Musculoskeletal Rehabilitation*, 27, 117–124.

Öztürk, G. T., Malas, F. U., Yldzgören, M. T., Baki, A. E., İnal, E. E., Batmaz, İ. I. İ., … Ozcakar, L. (2015). Ultrasonographic Assessment of the Femoral Cartilage Thickness in Patients with Pes Planus. *American Journal of Physical Medicine & Rehabilitation*, 94, 568–572.

Palmieri-Smith, R. M., Wojtys, E. M., & Potter, H. G. (2016). Early cartilage changes after anterior cruciate ligament injury: evaluation with imaging and serum biomarkers-a pilot study. *Arthroscopy*, 32, 1309–1318.

Pap, G., Eberhardt, R., Stürmer, I., Machner, A., Schwarzberg, H., Roessner, A., & Neumann, W. (1998). Development of osteoarthritis in the knee joints of Wistar rats after strenuous running exercise in a running wheel by intracranial self-stimulation. *Pathology, Research and Practice*, 194, 41–47.

Paradowski, P. T., Bergman, S., Sundén-Lundius, A., Lohmander, L. S., & Roos, E. M. (2006). Knee complaints vary with age and gender in the adult population. Population-based reference data for the Knee injury and Osteoarthritis Outcome Score (KOOS). *BMC Musculoskeletal Disorders*, 7.

Pate, R. R. (1995). Physical Activity and Public Health. *The Journal of the American Medical Association*, 273, 402-407.

Pearle, A. D., Warren, R. F., & Rodeo, S. A. (2005). Basic science of articular cartilage and osteoarthritis. *Clinics in Sports Medicine*, 24, 1–12.

Peat, G., Thomas, E., Handy, J., Wood, L., Dziedzic K., Myers, H., … Croft, P. (2006). The Knee Clinical Assessment Study-CAS(K). A prospective study of knee pain and knee osteoarthritis in the general population: baseline recruitment and retention at 18 months. *BMC Musculoskeletal Disorders*, 7.

Petersen, S. G., Saxne, T., Heinegard, D., Hansen, M., Holm, L., Koskinen, S., … Kjaer, M. (2010). Glucosamine but not ibuprofen alters cartilage turnover in osteoarthritis patients in response to physical training. *Osteoarthritis and Cartilage*, 18, 34–40.

Piscoya, J. L., Fermor, B., Kraus, V. B., Stabler, T. V., & Guilak, F. (2005). The influence of mechanical compression on the induction of osteoarthritis-related biomarkers in articular cartilage explants. *Osteoarthritis and Cartilage*, 13, 1092–1099.

Pitsillides, A. A., Will, R. K., Bayliss, M. T., & Edwards, J. C. (1994). Circulating and synovial fluid hyaluronan levels. Effects of intraarticular corticosteroid on the concentration and the rate of turnover. *Arthritis & Rheumatology*, 37, 1030–1038.

Pollard, T. C. B., Gwilym, S. E., & Carr, A. J. (2008). The assessment of early osteoarthritis. *The Bone & Joint Journal*, 90–B, 411–421.

Pols, M. A., Peeters, P. H., Bueno-De-Mesquita, H. B., Ocké, M. C., Wentink, C. A., Kemper, H. C., & Collette, H. J. (1995). Validity and repeatability of a modified Baecke questionnaire on physical activity. *International Journal of Epidemiology*, 24, 381–388.

Pruksakorn, D., Rojanasthien, S., Pothacharoen, P., Luevitoonvechkij, S., Wongtreratanachai, P., Ong-Chai, S., & Kongtawelert, P. (2009). Chondroitin sulfate epitope (WF6) and hyaluronic acid as serum markers of cartilage degeneration in patients following anterior cruciate ligament injury. *Journal of Science and Medicine in Sport*, 12, 445–448.

Pruksakorn, D., Tirankgura, P., Luevitoonvechkij, S., Chamnongkich, S., Sugandhavesa, N., Leerapun, T., & Pothacharoen, P. (2013). Changes in the serum cartilage biomarker levels of healthy adults in response to an uphill walk. *Singapore Medical Journal*, 54, 702–708.

Racunica, T. L., Teichtahl, A. J., Wang, Y., Wluka, A. E., English, D. R., Giles, G. G., … Cicuttini, F. M. (2007). Effect of physical activity on articular knee joint structures in community-based adults. *Arthritis & Rheumatology*, 57, 1261–1268.

Ratzlaff, C. R., Koehoorn, M., Cibere, J., & Kopec, J. A. (2012). Is lifelong knee joint force from work, home, and sport related to knee osteoarthritis? *International Journal of Rheumatology*, 2012, 584193.

Raynauld, J. (2003). Reliability of a quantification imaging system using magnetic resonance images to measure cartilage thickness and volume in human normal and osteoarthritic knees. *Osteoarthritis and Cartilage*, 11, 351–360.

Raynauld, J-P., Martel-Pelletier, J., Berthiaume, M-J., Beaudoin, G., Choquette, D., Haraoui, B., … Pelletier, J-P. (2006). Long term evaluation of disease progression through the quantitative magnetic resonance imaging of symptomatic knee osteoarthritis patients: correlation with clinical symptoms and radiographic changes. *Arthritis Research & Therapy*, 8, R21.

Rees, S. G., Davies, J. R., Tudor, D., Flannery, C. R., Hughes, C. E., Dent, C. M., & Caterson, B. (2002). Immunolocalisation and expression of proteoglycan 4 (cartilage superficial zone proteoglycan) in tendon. *Matrix Biology*, 21, 593–602.

Reijman, M., Pols, H. A., Bergink, A. P., Hazes, J. M., Belo, J. N., Lievense, A. M., & Bierma-Zeinstra, S. M. (2007). Body mass index associated with onset and progression of osteoarthritis of the knee but not of the hip: The Rotterdam Study. *Annals of the Rheumatic Diseases*, 66, 158–162.

Rhee, D. K., Marcelino, J., Baker, M., Gong, Y., Smits, P., Lefebvre, V., … Carpten, J. D. (2005). The secreted glycoprotein lubricin protects cartilage surfaces and inhibits synovial cell overgrowth. *Journal of Clinical Investigation*, 115, 622–631.

Ritter, S. Y., Collins, J., Krastins, B., Sarracino, D., Lopez, M., Losina, E., & Aliprantis, A. O. (2014). Mass spectrometry assays of plasma biomarkers to predict radiographic progression of knee osteoarthritis. *Arthritis Research & Therapy*, 16, 456.

Roos, H., Dahlberg, L., Hoerrner, L. A., Lark, M. W., Thonar, E. J., Shinmei, M., … Lohmander, L. S. (1995). Markers of cartilage matrix metabolism in human joint fluid and serum: the effect of exercise. *Osteoarthritis and Cartilage*, 3, 7–14.

Roughley, P. J., & Mort, J. S. (2014). The role of aggrecan in normal and osteoarthritic cartilage. *Journal of Experimental Orthopaedics*, 1,1-8.

Rousseau, J-C., Zhu, Y., Miossec, P., Vignon, E., Sandell, L. J., Garnero, P., & Delmas, P. D. (2004). Serum levels of type IIA procollagen amino terminal propeptide (PIIANP) are decreased in patients with knee osteoarthritis and rheumatoid arthritis. *Osteoarthritis and Cartilage*, 12, 440–447.

Russell, K. A., Palmieri, R. M., Zinder, S. M., & Ingersoll, C. D. (2006). Sex differences in valgus knee angle during a single-leg drop jump. *Journal of Athletic Training*, 41, 166–171.

Sasaki, E., Tsuda, E., Yamamoto, Y., Iwasaki, K., Inoue, R., Takahashi, I., … Ishibashi, Y. (2013). Serum hyaluronan levels increase with the total number of osteoarthritic joints and are strongly associated with the presence of knee and finger osteoarthritis. *International Orthopaedics*, 37, 925–930.

Sasaki, E., Tsuda, E., Yamamoto, Y., Maeda, S., Inoue, R., Chiba, D., … Ishibashi, Y. (2015). Serum hyaluronic acid concentration predicts the progression of joint space narrowing in normal knees and established knee osteoarthritis – a five-year prospective cohort study. *Arthritis Research & Therapy*, 17, 283.

Saxne, T., Heinegard, D., & Heinegård, D. (1992). Cartilage oligomeric matrix protein: a novel marker of cartilage turnover detectable in synovial fluid and blood. *British Journal of Rheumatology*, 31, 583–591.

Schmidt, T. A., Gastelum, N. S., Nguyen, Q. T., Schumacher, B. L., & Sah, R.L. (2007). Boundary lubrication of articular cartilage: Role of synovial fluid constituents. *Arthritis & Rheumatology*, 56, 882–891.

Schumacher, B. L., Schmidt, T. A., Voegtline, M. S., Chen, A. C., & Sah, R. L. (2005). Proteoglycan 4 (PRG4) synthesis and immunolocalization in bovine meniscus. *Journal of Orthopaedic Research*, 23, 562–568.

Seebeck, P., & Haima, P. (2013). Hyaluronic Acid (Hyaluronan) Biomarker for liver fibrosis and cirrhosis. *TECOmedical Clinical & Technical Review*, 1–16.

Segal, N. A., Findlay, C., Wang, K., Torner, J. C., & Nevitt, M. C. (2012). The longitudinal relationship between thigh muscle mass and the development of knee osteoarthritis. *Osteoarthritis and Cartilage*, 20, 1534–1540.

Segal, N. A., Glass, N. A, Torner, J., Yang, M., Felson, D. T., Sharma, L., … Lewis, C.E. (2010). Quadriceps weakness predicts risk for knee joint space narrowing in women in the MOST cohort. *Osteoarthritis and Cartilage*, 18, 769–775.

Senolt, L., Braun, M., Olejarova, M., Forejtova, S., Gatterova, J., & Pavelka, K. (2005). Increased pentosidine, an advanced glycation end product, in serum and synovial fluid from patients with knee osteoarthritis and its relation with cartilage oligomeric matrix protein. *Annals of the Rheumatic Diseases*, 64, 886–890.

Seo, S. K., Yang, H. I., Lim, K. J., Jeon, Y. E., Choi, Y. S., Cho, S., & Lee, B. S. (2012). Changes in serum levels of cartilage oligomeric matrix protein after estrogen and alendronate therapy in postmenopausal women. *Gynecologic and Obstetric Investigation*, 74, 143–150.

Sharif, M., George, E., Shepstone, L., Knudson, W., Thonar, E. J., Cushnaghan, J., & Dieppe, P. (1995a). Serum hyaluronic acid level as a predictor of disease progression in osteoarthritis of the knee. *Arthritis & Rheumatology*, 38, 760–767.

Sharif, M., Kirwan, J., Charni, N., Sandell, L. J., Whittles, C., & Garnero, P. (2007). A 5-yr longitudinal study of type IIA collagen synthesis and total type II collagen degradation in patients with knee osteoarthritis-association with disease progression. *Rheumatology (Oxford)*, 46, 938–943.

Sharif, M., Saxne, T., Shepstone, L., Kirwan, J. R., Elson, C. J., Heinegård, D., Dieppe, P. A., (1995b). Relationship between serum cartilage oligomeric matrix protein levels and disease progression in osteoarthritis of the knee joint. *British Journal of Rheumatology*, 34, 306–310.

Shimizu, M., Higuchi, H., Takagishi, K., Shinozaki, T., & Kobayashi, T. (2010). Clinical and biochemical characteristics after intra-articular injection for the treatment of osteoarthritis of the knee: prospective randomized study of sodium hyaluronate and corticosteroid. *Journal of Orthopaedic Science*, 15, 51–56.

Shin, K-A., Kim, A-C., Kim, Y-J., Lee, Y-H., Shin, Y-O., Kim, S-H., … Park, Y. (2012). Effect of Ultra-marathon (308 km) Race on Bone Metabolism and Cartilage Damage Biomarkers. *Annals of Physical and Rehabilitation Medicine*, 36, 80–87.

Shrier, I. (2004). Muscle dysfunction versus wear and tear as a cause of exercise related osteoarthritis: an epidemiological update. *British Journal of Sports Medicine*, 38, 526–535.

Shrout, P. E., & Fleiss, J. L. (1979). Intraclass correlations: uses in assessing rater reliability. *Psychological Bulletin*, 86, 420–428.

Sigal, R. J., Kenny, G. P., Boule, N. G., Wells, G. A., Prud, D., Fortier, M., … Jaffey, J. (2007). Effects of aerobic training, resistance training, or both on glycemic control in type 2 diabetes. *Annals of Internal Medicine*, 147, 357–369.

Singh, S., Kumar, D., Kumar, S., & Nishant, R. S. (2015). Cartilage Oligomeric Matrix Protein (COMP) and Hyaluronic Acid (HA): Diagnostic Biomarkers of Knee Osteoarthritis. *MedCrave Online Journal of Orthopaedics & Rheumatology*, 2, 999–1006.

Skou, S. T., & Aalkjaer, J. M. (2013). Ultrasonographic measurement of patellar tendon thickness--a study of intra- and interobserver reliability. *Clinical Imaging*, 37, 934–937.

Slauterbeck, J. R., Fuzie, S. F., Smith, M. P., Clark, R. J., Xu, T. K., Starch, D. W., Hardy, D. M. (2002). The menstrual cycle, sex hormones, and anterior cruciate ligament injury. *Journal of Athletic Training*, 37, 275–278.

Sniekers, Y. H., Weinans, H., van Osch, G. J. V. M., van Leeuwen, J. P. T. M. (2010). Oestrogen is important for maintenance of cartilage and subchondral bone in a murine model of knee osteoarthritis. *Arthritis Research & Therapy*, 12, R182.

Spector, T. D., Cicuttini, F.M., Baker, J., Loughlin, J., & Hart, D. (1996a). Genetic influences on osteoarthritis in women: a twin study. *British Medical Journal*, 312, 940–943.

Spector, T. D., Harris, P. A, Hart, D. J., Cicuttini, F. M., Nandra, D., Etherington, J., … Doyle, D. V. (1996b). Risk of osteoarthritis associated with long-term weight-bearing sports: a radiologic survey of the hips and knees in female ex-athletes and population controls. *Arthritis & Rheumatology*, 39, 988–995.

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(9):769–81.

Stammberger, T., Eckstein, F., Englmeier, K. H., & Reiser, M. (1999). Determination of 3D cartilage thickness data from MR imaging: computational method and reproducibility in the living. *Magnetic Resonance in Medicine*, 41, 529–536.

Strasser, B. (2013). Physical activity in obesity and metabolic syndrome. *Annals of the New York Academy of Sciences*, 1281, 141–159.

Sun, Y., Berger, E. J., Zhao, C., An, K-N., Amadio, P. C., & Jay, G. (2006). Mapping lubricin in canine musculoskeletal tissues. *Connective Tissue Research*, 47, 215–221.

Swann, D. A., Slayter, H. S., & Silver, F.H. (1981). The molecular structure of lubricating glycoprotein-I, the boundary lubricant for articular cartilage. *Journal of Biological Chemistry*, 256, 5921–5925.

Tanamas, S., Hanna, F. S., Cicuttini, F. M., Wluka, A. E., Berry, P., & Urquhart, D. M. (2009). Does knee malalignment increase the risk of development and progression of knee osteoarthritis? A systematic review. *Arthritis & Rheumatology*, 61, 459–467.

Tarhan, S., Unlu, Z., & Goktan, C. (2003). Magnetic resonance imaging and ultrasonographic evaluation of the patients with knee osteoarthritis: a comparative study. *Clinical Rheumatology*, 22, 181–188.

Thomas, J. R., & Nelson, K. J. (1990). *Research methods in physical activity*. Champaign, IL: Human Kinetics.

Urquhart, D. M., Tobing, J. F. L., Hanna, F. S., Berry, P., Wluka, A. E., Ding, C., & Cicuttini, F. M. (2011). What is the effect of physical activity on the knee joint? A systematic review. *Medicine & Science in Sports & Exercise*, 43, 432–442.

Ushiyama, T., Ueyama, H., Inoue, K., Ohkubo, I., & Hukuda, S. (1999). Expression of genes for estrogen receptors alpha and beta in human articular chondrocytes. *Osteoarthritis and Cartilage*, 7, 560–566.

Van Ginckel, A., Baelde, N., Almqvist, K. F., Roosen, P., McNair, P., & Witvrouw, E. (2010). Functional adaptation of knee cartilage in asymptomatic female novice runners compared to sedentary controls. A longitudinal analysis using delayed Gadolinium Enhanced Magnetic Resonance Imaging of Cartilage (dGEMRIC). *Osteoarthritis and Cartilage*, 18, 1564–1569.

Van Spil, W. E., DeGroot, J., Lems, W. F., Oostveen, J. C. M., & Lafeber, F. P. J. G. (2010). Serum and urinary biochemical markers for knee and hip-osteoarthritis: a systematic review applying the consensus BIPED criteria. *Osteoarthritis and Cartilage*, 18, 605–612.

Vanwanseele, B., Eckstein, F., Knecht, H., Stüssi, E., & Spaepen, A. (2002). Knee cartilage of spinal cord-injured patients displays progressive thinning in the absence of normal joint loading and movement. *Arthritis & Rheumatology*, 46, 2073–2078.

Verma, P., & Dalal, K. (2013). Serum cartilage oligomeric matrix protein (COMP) in knee osteoarthritis: a novel diagnostic and prognostic biomarker. *Journal of Orthopaedic Research*, 31, 999–1006.

Vignon, É., Valat, J. P., Rossignol, M., Avouac, B., Rozenberg, S., Thoumie, P., … Hilliquin, P. (2006). Osteoarthritis of the knee and hip and activity: a systematic international review and synthesis (OASIS). *Joint Bone Spine*, 3, 442–455.

Vilim, V., Olejarova, M., Machacek, S., Gatterova, J., Kraus, V. B., Pavelka, K., & Vilím, V, (2002). Serum levels of cartilage oligomeric matrix protein (COMP) correlate with radiographic progression of knee osteoarthritis. *Osteoarthritis and Cartilage*, 10, 707–713.

Wakitani, S., Nawata, M., Kawaguchi, A., Okabe, T., Takaoka, K., Tsuchiya, T., … Miyazaki, K. (2007). Serum keratan sulfate is a promising marker of early articular cartilage breakdown. *Rheumatology (Oxford)*, 46, 1652–1656.

Wakitani, S., Okabe, T., Kawaguchi, A., Nawata, M., & Hashimoto, Y. (2010). Highly sensitive ELISA for determining serum keratan sulphate levels in the diagnosis of OA. *Rheumatology (Oxford)*, 49, 57–62.

Waller, B., Munukka, M., Multanen, J., Rantalainen, T., Pöyhönen, T., Nieminen, M. T., … Heinonen, A. (2013). Effects of a progressive aquatic resistance exercise program on the biochemical composition and morphology of cartilage in women with mild knee osteoarthritis: protocol for a randomised controlled trial. *BMC Musculoskeletal Disorders*, 14, 82.

Wang, Y., Wluka, A. E., Jones, G., Ding, C., & Cicuttini, F. M. (2012). Use magnetic resonance imaging to assess articular cartilage. *Therapeutic Advances in Musculoskeletal. Disease*, 4, 77–97.

Whaley, M. H., Brubaker, P. H., Otto, R. M., & Armstrong, L. E. (2006). Health-related physical testing and interpretation. In: ACSM's Guidelines for Exercise Testing and Prescription. Balimore, MA: Lippincott, Williams and Wilkins.

Williams, D., Kuipers, A., Mukai, C., & Thirsk, R. (2009). Acclimation during space flight: effects on human physiology. *Canadian Medical Association*, 180, 1317–1323.

Wilson, M. G., Michet, C. J., Ilstrup, D. M., & Melton, L. J. (1990). Idiopathic symptomatic osteoarthritis of the hip and knee: a population-based incidence study. *Mayo Clinic Proceedings*, 65, 1214–1221.

Wisłowska, M., & Jabłońska, B. (2005). Serum cartilage oligomeric matrix protein (COMP) in rheumatoid arthritis and knee osteoarthritis. *Clinical Rheumatology*, 24, 278–284.

Woolf, A.D., & Pfleger, B. (2003). Burden of major musculoskeletal conditions. *Bulletin of the World Health Organization*, 81, 646–656.

Yoon, C-H., Kim, H-S. H-Y., Ju, J. H., Jee, W-H., & Park, S-H. (2008). Validity of the sonographic longitudinal sagittal image for assessment of the cartilage thickness in the knee osteoarthritis. *Clinical Rheumatology*, 27, 1507–1516.

Young, A. A., McLennan, S., Smith, M. M., Smith, S. M., Cake, M. A., Read, R. A., … Little, C. B. (2006). Proteoglycan 4 downregulation in a sheep meniscectomy model of early osteoarthritis. *Arthritis Research & Therapy*, 8, R41.

10 Appendices

10.1 Appendix A - 7-day International Physical Activity Questionnaire (IPAQ) (long version)

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will 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. Please 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.

Think about all the **vigorous** and **moderate** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal.

The first section is about your work. This includes paid jobs, farming, volunteer work, course work, and any other unpaid work that you did outside your home. Do not include unpaid work you might do around your home, like housework, yard work, general maintenance, and caring for your family. These are asked in Part 3.

PART 1: JOB-RELATED PHYSICAL ACTIVITY

1. Do you currently have a job or do any unpaid work outside your home?

   ☐ Yes

   ☐ No → ***Skip to PART 2: TRANSPORTATION***

The next questions are about all the physical activity you did in the **last 7 days** as part of your paid or unpaid work. This does not include traveling to and from work.

2. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, heavy construction, or climbing up stairs **as part of your work**? Think about only those physical activities that you did for at least 10 minutes at a time.

   _____ **days per week**

   ☐ No vigorous job-related physical activity → ***Skip to question 4***

3. How much time did you usually spend on one of those days doing **vigorous** physical activities as part of your work?

   _____ **hours per day**
   _____ **minutes per day**

4. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads as part of your work? Please do not include walking.

_____ **days per week**

☐ No moderate job-related physical activity → ***Skip to question 6***

5. How much time did you usually spend on one of those days doing **moderate** physical activities as part of your work?

_____ **hours per day**
_____ **minutes per day**

6. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **as part of your work**? Please do not count any walking you did to travel to or from work.

_____ **days per week**

☐ No job-related walking → ***Skip to PART 2: TRANSPORTATION***

7. How much time did you usually spend on one of those days **walking** as part of your work?

_____ **hours per day**
_____ **minutes per day**

PART 2: TRANSPORTATION PHYSICAL ACTIVITY

These questions are about how you traveled from place to place, including to places like work, stores, movies, and so on.

8. During the **last 7 days**, on how many days did you **travel in a motor vehicle** like a train, bus, car, or tram?

_____ **days per week**

☐ No traveling in a motor vehicle → ***Skip to question 10***

9. How much time did you usually spend on one of those days **traveling** in a train, bus, car, tram, or other kind of motor vehicle?

_____ **hours per day**
_____ **minutes per day**

Now think only about the **bicycling** and **walking** you might have done to travel to and from work, to do errands, or to go from place to place.

10. During the **last 7 days**, on how many days did you **bicycle** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No bicycling from place to place → ***Skip to question 12***

11. How much time did you usually spend on one of those days to **bicycle** from place to place?

_____ **hours per day**
_____ **minutes per day**

12. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time to go **from place to place**?

_____ **days per week**

☐ No walking from place to place → ***Skip to PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY***

13. How much time did you usually spend on one of those days **walking** from place to place?

_____ **hours per day**
_____ **minutes per day**

PART 3: HOUSEWORK, HOUSE MAINTENANCE, AND CARING FOR FAMILY

This section is about some of the physical activities you might have done in the **last 7 days** in and around your home, like housework, gardening, yard work, general maintenance work, and caring for your family.

14. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, chopping wood, shoveling snow, or digging **in the garden or yard**?

_____ **days per week**

☐ No vigorous activity in garden or yard → ***Skip to question 16***

15. How much time did you usually spend on one of those days doing **vigorous** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

16. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, sweeping, washing windows, and raking **in the garden or yard**?

_____ **days per week**

☐ No moderate activity in garden or yard → ***Skip to question 18***

17. How much time did you usually spend on one of those days doing **moderate** physical activities in the garden or yard?

_____ **hours per day**
_____ **minutes per day**

18. Once again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** activities like carrying light loads, washing windows, scrubbing floors and sweeping **inside your home**?

_____ **days per week**

☐ No moderate activity inside home → ***Skip to PART 4: RECREATION, SPORT AND LEISURE-TIME PHYSICAL ACTIVITY***

19. How much time did you usually spend on one of those days doing **moderate** physical activities inside your home?

_____ **hours per day**
_____ **minutes per day**

PART 4: RECREATION, SPORT, AND LEISURE-TIME PHYSICAL ACTIVITY

This section is about all the physical activities that you did in the **last 7 days** solely for recreation, sport, exercise or leisure. Please do not include any activities you have already mentioned.

20. Not counting any walking you have already mentioned, during the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time **in your leisure time**?

_____ **days per week**

☐ No walking in leisure time → ***Skip to question 22***

21. How much time did you usually spend on one of those days **walking** in your leisure time?

_____ **hours per day**
_____ **minutes per day**

22. Think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **vigorous** physical activities like aerobics, running, fast bicycling, or fast swimming **in your leisure time**?

_____ **days per week**

☐ No vigorous activity in leisure time → ***Skip to question 24***

23. How much time did you usually spend on one of those days doing **vigorous** physical activities in your leisure time?

_____ **hours per day**
_____ **minutes per day**

24. Again, think about only those physical activities that you did for at least 10 minutes at a time. During the **last 7 days**, on how many days did you do **moderate** physical activities like bicycling at a regular pace, swimming at a regular pace, and doubles tennis **in your leisure time**?

_____ **days per week**

☐ No moderate activity in leisure time → ***Skip to PART 5: TIME SPENT SITTING***

25. How much time did you usually spend on one of those days doing **moderate** physical activities in your leisure time?
_____ **hours per day**
_____ **minutes per day**

PART 5: TIME SPENT SITTING

The last questions are about the time you spend sitting while 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. Do not include any time spent sitting in a motor vehicle that you have already told me about.

26. During the **last 7 days**, how much time did you usually spend **sitting** on a **weekday**?

_____ **hours per day**
_____ **minutes per day**

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

_____ hours per day
_____ minutes per day

This is the end of this questionnaire, thank you for participating.

10.2 Appendix B - Measurement of a Person's Habitual Physical Activity questionnaire

MEASUREMENT OF A PERSON'S HABITUAL PHYSICAL ACTIVITY

Overview:

This questionnaire evaluates your physical activity ***over the last year*** by separating it into three distinct dimensions.

(1) work activity, (2) sports activity, and (3) leisure activity. Please answer or circle your choice as appropriate. Also, please remember to consider your activity over the last 12 months only.

Questions	**Answer**
PART 2: WORK RELATED ACTIVITY	
1. What is your main occupation? ...	
2. At work I sit...	Never / seldom / sometimes / often / always
3. At work I stand ...	Never / seldom / sometimes / often / always
4. At work I walk ...	Never / seldom / sometimes / often / always
5. At work I lift heavy loads ...	Never / seldom / sometimes / often / always
6. After work I am tired ...	Never / seldom / sometimes / often / always
7. At work I sweat ...	Never / seldom / sometimes / often / always
8. In comparison with others of my own age I think my work is physically ...	much lighter / lighter / as heavy / heavier / much heavier

PART 2: SPORT RELATED ACTIVITY

9. Do you play sport? yes/no
If yes:
- which sport do you play most frequently? ...
- how many hours a week? < 1 / 1 -2 / 2-3 / 3-4 / >4
- how many months a year? > 1 / 1-3 / 4-6 / 7-9 / >9

If you play a second sport
- which sport is it?….
- how many hours a week? < 1 / 1 -2 / 2-3 / 3-4 / >4
- how many months a year? > 1 / 1-3 / 4-6 / 7-9 / >9

10. In comparison with others of my own age I think my physical activity during leisure time is ...
much less / less / the same / more / much more

11. During leisure time I sweat ...
Never / seldom / sometimes / often / very often

12. During leisure time I play sport ...
Never / seldom / sometimes / often / very often

PART 3: LEISURE RELATED ACTIVITY

13. During leisure time I watch television ...
Never / seldom / sometimes / often / very often

14. During leisure time I walk ...
Never / seldom / sometimes / often / very often

15. During leisure time I cycle ...
Never / seldom / sometimes / often / very often

16. How many minutes per day do you walk and/or cycle to and from work, school and shopping?
<5 / 5-15 / l5-30 / 30-45 / >45

17. During leisure time I do do-it-yourself (DIY) activities ...
Never / seldom / sometimes / often / very often

18. During leisure time I work in the garden ...
Never / seldom / sometimes / often / very often

19. How many hours per day do you sleep on average?
≤5 / 6-7-8 /≥ 9

This is the end of the questionnaires, thank you for participating.

10.3 Appendix C - Participant medical questionnaire

Bangor University
SCHOOL OF SPORT, HEALTH AND EXERCISE SCIENCES

Name of Participant ……..……
Age ……

Are you in good health? ☐ YES ☐ NO

If no, please explain

How would you describe your present level of activity?
Tick intensity level and indicate approximate duration.

Vigorous		Moderate		Low intensity	

Duration (minutes)…….

How often?

< Once per month		2-3 times per week	
Once per month		4-5 times per week	
Once per week		> 5 times per week	

Have you suffered from a serious illness or accident? ☐ YES ☐ NO

If yes, please give particulars:

Do you suffer from allergies? ☐ YES ☐ NO

If yes, please give particulars:

Do you suffer, or have you ever suffered from:

	YES	NO		YES	NO
Asthma			Epilepsy		
Diabetes			High blood pressure		
Bronchitis					

Are you currently taking medication? ☐ YES ☐ NO

If yes, please give particulars:

Are you currently attending your GP for any condition or have you consulted your doctor in the last three months? ☐ YES ☐ NO

If yes, please give particulars:
Have you, or are you presently taking part in any other laboratory experiment?

☐ YES ☐ NO

PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to do the experimental exercise task if they:

- have a fever, cough or cold, or suffer from fainting spells or dizziness;
- have suspended training due to a joint or muscle injury;
- have a known history of medical disorders, i.e. high blood pressure, heart or lung disease;
- have had hyper/hypothermia, heat exhaustion, or any other heat or cold disorder;
- have anaphylactic shock symptoms to needles, probes or other medical-type equipment;
- have chronic or acute symptoms of gastrointestinal bacterial infections (e.g. Dysentery, Salmonella);
- have a history of infectious diseases (e.g. HIV, Hepatitis B); and if appropriate to the study design, have a known history of rectal bleeding, anal fissures, haemorrhoids, or any other condition of the rectum.

PLEASE COMPLETE AND SIGN THE DECLARATION BELOW

DECLARATION

I agree that I have none of the above conditions and I hereby volunteer to be a participant in experiments/investigations during the period of ……20……

My replies to the above questions are correct to the best of my belief and I understand that they will be treated with the strictest confidence. The experimenter has explained to my satisfaction the purpose of the experiment and possible risks involved.

I understand that I may withdraw from the experiment at any time and that I am under no obligation to give reasons for withdrawal or to attend again for experimentation.

Furthermore, if I am a student, I am aware that taking part or not taking part in this experiment, will neither be detrimental to, or further, my position as a student.

I undertake to obey the laboratory/study regulations and the instructions of the experimenter regarding safety, subject only to my right to withdraw declared above.

Signature *(participant)* …….. Date ……..

Print name ……..

Signature *(experimenter)* …… Date ……..

Print name ……..…

10.4 Appendix D – Radar graph plot

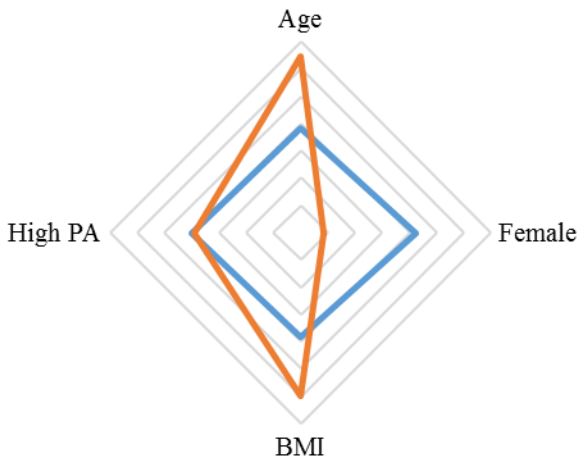


Figure 1. Radar plot to highlight participant characteristics of clear (blue) and unmeasurable (orange) ultrasound images. Radar spoke represent mean z-scores. High PA = High levels of physical activity (i.e. ≥ 5 times / week).

10.5 Appendix E – Medical, lifestyle, injury and physical activity questionnaire

PRIFYSGOL BANGOR UNIVERSITY

ID NUMBER (For researcher):	
NAME:	D.O.B (AGE):
ADDRESS:	GENDER: male / female
POSTCODE:	HEIGHT:
TELEPHONE NO:	WEIGHT:
OCCUPATION:	

1. Are you in good health (*If no, please explain*)? ☐ **YES** ☐ **NO**

……

2. Are you currently, or have you been recently, taking any medication, supplements or vitamins (*If yes, please give particulars. This includes anti-inflammatory drugs such as ibuprofen*)? ☐ **YES** ☐ **NO**

……

3. Are you currently attending your GP for any condition or have you consulted your doctor in the last three months (*If yes, please give particulars*)? ☐ **YES** ☐ **NO**

……

4. Within the past six months have you suffered from a serious illness, injury or accident (*If yes, please give particulars*)? ☐ **YES** ☐ **NO**

……

5. Do you suffer from, or have you ever suffered from any of the following?

A heart condition	☐ **YES**	☐ **NO**
Chest pain during exercise	☐ **YES**	☐ **NO**
Chest pain at rest	☐ **YES**	☐ **NO**
Dizziness or loss of consciousness	☐ **YES**	☐ **NO**
Asthma or bronchial problems	☐ **YES**	☐ **NO**
Arthritis: Rheumatoid / osteoarthritis	☐ **YES**	☐ **NO**
Epilepsy	☐ **YES**	☐ **NO**
Joint or muscle injury	☐ **YES**	☐ **NO**

6. Do you know any reason why you should not do physical activity? ☐ **YES** ☐ **NO**

7. Study specific exclusion criteria – do you have a history of the following?

A knee related injury within the last 5 years ☐ **YES** ☐ **NO**

Knee malalignment (varus / valgus) greater than 15° ☐ **YES** ☐ **NO**

Women only:

8. Study specific exclusion criteria

Are you pregnant? ☐ **YES** ☐ **NO**

Are you pre-menopausal? ☐ **YES** ☐ **NO**

PLEASE READ THE FOLLOWING CAREFULLY

Persons will be considered unfit to take part in the experimental study if they have answered yes to questions 5, 6, 7 or 8

If you are in any doubt after completing this questionnaire, please consult your doctor prior to physical activity.

Participant Signature …….. Date: ……

Print name **……**

Researcher Signature …….. Date: ……..

Print name **……**

Lifestyle questionnaire

1. Are you a smoker?

1 = yes, regularly (Go to Q2)

2 = occasionally (Go to Q3)

3 = no (Go to Q4)

2. On average, how many cigarettes do you smoke a day? _____

3. On how many days a week do you smoke cigarettes?

1 = usually on one day or less

2 = usually on 2 to 4 days

3 = almost every day

4. Did you ever smoke cigarettes regularly in the past?

1 = yes (Go to Q5)

2 = no (Go to Q6)

5. When did you stop smoking cigarettes regularly? Year ……..

If in the last 12 months

1 = less than 1 month ago

2 = 1-6 months ago

3 = 6-12 months ago

6. Do you consume alcohol regularly? ☐ YES ☐ NO

How many units per week _____

Physical activity questionnaire

1. **Are you currently engaged in regular physical activity? YES / NO**

   If NO, please move to Question 2. If YES, please provide brief details of:

   a. The type of physical activity:

      ……..…….

   b. How often do you exercise on a weekly basis?

      ……

   c. Current number of hours you spend exercising (hours/week)?

      .....……

   d. How would you describe the typical intensity of training (*Tick intensity level)?*

      HIGH ☐ MODERATE ☐ LOW INTENSITY ☐

   e. How many years have you been engaged in regular physical activity?

      .....……

2. **Have you previously been engaged in regular physical activity? YES / NO**

   a. What type of physical activity were you involved in:

      ……..……

   b. Approximately how many years were you involved for:

      ……..……

   c. How often did you exercise on a weekly basis?

      ……..……

   d. How many hours did you spend exercising (hours/week)?

      ……..……

   e. How would you describe the typical intensity of your previous physical activity (*Tick intensity level)?*

      HIGH ☐ MODERATE ☐ LOW INTENSITY ☐

   f. Were you considered more active than others of your age and sex?

      During Adolescence 13-18 years Yes / No

      During Young adulthood 19-35 years Yes / No

      During Middle adulthood 36-50 years Yes / No

      During years 50+ Yes / No

Knee-related questionnaire: problems, injury and overuse

1. **Is your right or left side your dominant side (if you are unsure, please think about which side you would naturally kick a ball with)?**

   .....

2. **Have you ever suffered a knee injury? If yes, please specify.**
   - Details:
   - Right or Left side (please specify)
   - When / how long ago?
   - How painful was it (from 0 to 10, with 10 being the most pain)?
   - How did it affect your physical activity?
   - Did it stop you from engaging in physical activity for more than 2 weeks?
   - ☐ YES ☐ NO If yes, please specify:

3. **Do you currently, or have you ever suffered from chronic knee pain? If yes, please specify.**
   - Details (e.g. pain, stiffness, swelling, giving way):
   - When / how long ago?
   - How painful was it (from 0 to 10, with 10 being the most pain)?
   - How did it affect your physical activity?
   - Did it stop you from engaging in physical activity for more than 2 weeks?
4. **Do you have other family members that have had chronic knee pain or knee osteoarthritis?**
   - ☐ YES ☐ NO If yes, please specify:
5. **Do you take medications to prevent knee pain? If yes, please specify.**

   e.g. paracetamol, ibuprofen
6. **Do you take other remedies or see a health professional on a regular basis to prevent knee pain? If yes, please specify.**
7. e.g. fish oils, glucosamine, herbal remedies, acupuncture, chiropractor, physiotherapist, copper bracelets

10.6 Appendix F - ACSM risk stratification table

Table 1. Coronary artery disease ACSM risk stratification threshold

Risk Factor	Defining Criteria
Age	Men ≥ years; Women ≥ 55 years
Family history	Heart attack, 'bypass surgery', or sudden death before age of 55 years for father / brother, or before 65 years for mother/ sister
Cigarette smoking	Current smoker, or quit < 6 months ago
Sedentary lifestyle	Not participating in moderate levels of physical activity at least 3 times per week for period of at least 3 months or more
Obesity	Body mass index ≥ 30 kg/m^2 or waist girth > 102 cm for men and > 88 cm for women
Hypertension	Systolic blood pressure ≥ 140 mmHg and / or diastolic greater ≥ 90 mmHg, or taking medication.
Dyslipidemia	LDL > 130 mg/dl or HDL < 40 mg/dl, or taking medication. Or TG > 200 mg/dl
Pre-diabetes	IFG ≥ 100 mg/dl or OGTT ≥ 140 and ≤ 190 mg/dl confirmed by two different measurements

10.7 Appendix G - The comparison of femoral cartilage thickness and serum biomarkers among previously injured and non-injured healthy men

Purpose

Several studies have reported an increased prevalence of OA following knee injury (Muthuri et al., 2011). However, whether the increased risk of knee OA following injury manifests in early changes in cartilage thickness and serum biomarker profiles remain largely unclear. Traumatic acute knee injuries such as cruciate and meniscal injuries are associated with increases in serum COMP concentrations (Kuhne et al., 2014; Palmieri-Smith et al., 2016), which can remain elevated for prolonged periods (Kuhne et al., 2014), but usually return to within normal healthy values following extended recovery (Åhlén et al., 2015; Cattano et al., 2016). Whether previous injury affects femoral cartilage thickness or other serum biomarkers such as HA and serum lubricin remains unknown.

Methods

A sub-sample of participants who had previously reported an injury in Chapter 5 (n =13) were compared with a group of non-injured participants (n = 13) matched for age, weight and fitness level. All knee related injuries reported within this study were sustained at least 5 years prior to the participant's initial visit. Participants were required to report a knee injury if they had visited a medical professional and had needed to stop physical activity for a period of 2 weeks or more. However, due to the retrospective nature of this questionnaire, the specific details of the injury were often unknown. Anthropometrics, VO_2max, and serum COMP, HA and lubricin were assessed as outlined in Chapter 5. To assess the potential differences in femoral cartilage thickness, individuals (n =7) with an injury to a single knee were compared to the non-injured side.

Statistical analyses

Independent t-tests were used to determine between groups differences (injured vs non injured) for all variables. For the multiple comparisons, bonferroni corrections were used for statistical significance.

Results

The between-group analysis of a group of previously injured individuals and a group non-injured individuals did not reveal any statistical differences in physical characteristics, VO_2max, or serum biomarker concentration (Table 1; all $P > 0.05$). In previously injured individuals, a comparison of femoral cartilage thickness measured on the injured side compared with the non-injured side, did not demonstrate a significant difference (Table 2).

The greatest difference in cartilage thicknesses measured between injured and non-injured sides was at the lateral condyle (injured: 1.71 mm vs non-injured: 2.09 mm; P = 0.08).

Table 1 Data from a group of participants who had reported a previous knee injury and a group of non-injured participants matched for age, weight and fitness level.

Variable	**Injured**	**(n = 13)**	**Non-injured**	**(n = 13)**	**P-value**
	Mean	**SD**	**Mean**	**SD**	
Age (years)	53	15	52	14	0.808
Height (metres)	1.74	0.61	1.79	0.70	0.116
Weight (kg)	81.2	8.6	83.6	11.8	0.553
BMI	26.7	2.3	26.3	3.6	0.695
VO^2max (ml/kg/min)	39	9	37	8	0.616
COMP (log)	6.55	0.25	6.61	0.16	0.590
Lubricin (ng/ml)	106.17	39.29	127.72	32.52	0.152
HA (ng/ml)	23.80	23.26	27.51	21.35	0.479

Injury detail: Injuries were 'old', i.e. sustained between 5-49 years; all involved rest for a minimum of 2 weeks; injuries were typically sporting injuries to menisci or ligaments. (n = 2 sustained playing football; n = 3 sustained skiing).

Table 2 Data from participants who had previously sustained a knee injury to a single side; femoral cartilage thickness comparison of injured and non-injured side.

Cartilage thickness (mm)	**Injured Side**	**(n = 7)**	**Non-injured side**	**(n = 7)**	**P - value**
	Mean	**SD**	**Mean**	**SD**	
Intercondyle notch	2.28	0.35	2.31	0.66	0.91
Lateral condyle	1.71	0.19	2.09	0.40	0.08
Medial condyle	1.77	0.22	2.10	0.44	0.10

Conclusions

This analysis revealed no differences in the serum biomarker profile of previously injured and non-injured individuals and is comparable to previous studies exploring serum COMP (Catterall et al., 2010; Lohmander et al., 1989) and serum HA (Pruksakorn et al., 2009). Moreover, although some studies have reported elevated serum COMP following injury (Kuhne et al., 2014; Palmieri-Smith et al., 2016; Wakitani et al., 2007), serum biomarkers often return to normal concentrations with recovery (Åhlén et al., 2015; Cattano et al., 2016). Therefore, acute injury may to place the knee joint at increased short-term risk (as evidenced by a change in the biochemical milieu), which is subsequently reduced with recovery time. Support for this is provided by research that has shown that following appropriate rehabilitation, inflammatory cytokines and biomarkers of cartilage metabolism between injured and non-injured sides appear to be unchanged 8 years post injury (Ahlen et al., 2015). In addition, the results of the present study did not reveal any significant differences in femoral cartilage thickness between the injured and non-injured knee, although results demonstrated a trend towards reduced lateral femoral condyle thickness in the injured side compared to the non-injured side ($P = 0.08$). Overall, in healthy individuals, a previous knee injury is not associated with long term differences in the profile of serum biomarkers or femoral cartilage thickness. However, given that injury is a significant risk factor of future OA, future studies should explore whether potential differences in the serum biomarker profile immediately post exercise relate to future OA.