A RADIOLOGICAL INVESTIGATION OF KNEE OSTEOARTHRITIS

ANALYSES OF MINIMUM JOINT SPACE WIDTH, BONE MINERAL DENSITY AND ARTICULAR CARTILAGE VOLUME AND THICKNESS IN AND AROUND THE KNEE JOINT IN HEALTHY INDIVIDUALS AND THOSE WITH KNEE OSTEOARTHRITIS

By

KAREN A. BEATTIE, B.Sc.

A Thesis

Submitted to the School of Graduate Studies
In Partial Fulfillment of the Requirements
for the Degree

Doctor of Philosophy

McMaster University

DOCTOR OF PHILOSOPHY (2006) McMaster University
(Medical Sciences) Hamilton, Ontario

TITLE: Analyses of Minimum Joint Space Width, Bone Mineral Density and Articular Cartilage Volume and Thickness in and around the Knee Joint in Healthy Individuals and Those with Knee Osteoarthritis

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NUMBER OF PAGES: 236

Abstract

The task of evaluating tissue parameters in and around the knee joint in those with and without knee osteoarthritis was taken upon in this thesis. One knee X-ray, a Dual X-ray Absorptiometry scan of the lumbar spine, hip, distal femur and proximal tibia and an MRI scan of the knee were acquired from 120 healthy and 55 osteoarthritic participants 20 to 83 years of age. Scans were analyzed to yield outcome parameters of minimum joint space width (mJSW), bone mineral density (BMD) and cartilage volume and thickness using dedicated software algorithms. All osteoarthritic individuals completed WOMAC and SF-36 questionnaires.

Joint space analyses revealed that healthy males have larger values than females of similar ages and that increasing disease severity is reflected by joint space narrowing. Data suggests there may be a threshold mJSW value for both males and females at which point one could be identified with early knee OA. Radiographic measures were also found to correlate with symptoms of pain (in females) and physical function.

Cartilage volume and thickness analyses generally paralleled those of mJSW. Measurements appeared constant throughout the ages in healthy males while a small decline in parameters was apparent in healthy females. Increasing disease severity, body mass index and age were also significantly related to joint space narrowing. Cartilage volume and thickness were significantly correlated with pain, stiffness and physical function in those with knee OA.

In healthy and osteoarthritic individuals, BMD measurements in the distal femur and proximal tibia were higher in males than females but were not significantly different in those with knee OA versus those without. Disease severity was found to be positively correlated with subchondral BMD in the distal femur and the proximal tibia suggesting that those with more severe disease may have more sclerotic bone compared to those with milder disease.

Acknowledgments

The journey to the completion of this degree has been a long one, just like a long road trip with a known destination and a map guiding the path but without knowing the road and the weather conditions along the way. There have been times when the road has split into two with a choice having to be made about which to take, knowing of course that each would lead me to the same place in the end. Sometimes the road has been paved and straight while at other times it has been rocky and bumpy, twisting and winding. I have made many stops along the road to regain my composure, taking time to learn more about myself, and at those stops I have taken time to breath and count my blessings. For the most part, the blessings have been the people in my life who have helped me choose the shorter, smoother or straighter path. More importantly, though, these same people made the journey this way when I chose or came upon the bumps and twists and turns, most often when I forgot what the final destination would be or lost sight of it altogether.

To Rick, you are the best supervisor I could have dreamed of having when I entered graduate school. You have provided me with opportunities that I never thought were possible. You gave me confidence when I needed a boost and you calmed me down at times of anxiety. You trusted me and gave me independence to do and work as you expected but you never failed to keep site of the big picture and ask me about my life. You have inspired me to achieve my goals and have showed me that leading by example is the most profound way of teaching and supervising.

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TABLE OF CONTENTS

Tables

Figures

A RADIOLOGICAL INVESTIGATION OF KNEE OSTEOARTHRITIS

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TABLE OF CONTENTS

Tables

Figures

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Chapter 1: Introduction

Osteoarthritis (OA) is the most common form of arthritis and has been defined by Altman as "a heterogeneous group of conditions that lead to joint symptoms and signs which are associated with defective integrity of articular cartilage, in addition to related changes in the underlying bone and at the joint margins" (1;2). The causes of OA may be idiopathic, mostly related to aging, or secondary, as in congenital abnormalities, response to trauma or diseases such as gout or pseudogout (3). Although articular cartilage degeneration or loss is seemingly the signature event in OA, it represents only a piece of the puzzle with regards to the structural changes which accompany this complex multifactorial disease. Known to affect the synovial joints, the condition is often also associated with concomitant changes in soft tissue structures surrounding the joint such as the synovium and joint capsule (4). While some once considered OA to be a disease of wear and tear and a natural consequence of aging, the existence of elderly individuals without radiographic or symptomatic disease has shown that this is not the case (1;5). The paradigm has since shifted to a view of a disease that appears, as described by Sowers, to be a mechanically driven, chemically mediated disease process in which there is attempted joint repair (5-7). Figures 1 and 2 represent similar hypothesized pathophysiological pathways of OA as described by Felson and Loeser (1;5).

Many epidemiological studies have been conducted to investigate the incidence, prevalence and risk factors of knee OA. Unfortunately, such rates differ depending on variable diagnostic definitions of OA. For example, estimated prevalences of knee OA determined on the basis of patient-reports, clinician-diagnosed or radiographic presence of disease can vastly differ between studies. Clinically speaking, the most compelling definition of joint disease is likely one which incorporates both radiographic/pathological features as well as symptoms (4). Regardless of the definition of OA employed in different studies, two observations consistently predominate; 1) there is a clear association between the prevalence of knee OA and aging and 2) there is a higher prevalence of knee OA among females as compared to males (4;6).

According to data from the Framingham study, 33% of individuals ≥60 years of age showed radiographic evidence of knee OA as defined by the Kellgren-Lawrence scale (8). In females, the prevalence of knee OA increased significantly with aging with prevalences of 25.1% in those <70 years, 36.3% in those 70-79 years and 52.6% in those ≥ 80 years of age. In males, the prevalence of knee OA also increased with age, albeit only slightly. Of those males <70 years, 30.4% had radiographic knee OA compared to 30.7% in those 70-79 years and 32.6% in those ≥ 80 years of age. Other epidemiological studies have also found gender disparities in the prevalence of knee OA at an even greater degree than was identified

in the Framingham study (9;10). The higher prevalence of knee OA in females and the trend towards a rather dramatic increase in the prevalence of knee OA in aging females has led to the speculation that estrogen may play a role in the pathophysiology of the disease with estrogen exerting potential chondroprotective effects prior to menopause which may recede post-menopause (11;12). It should be noted that prior to age 50 years, men appear to have a higher prevalence of OA than do women, likely because of the levels of estrogen in females before menopause and also because of the role of athletics and injury in the male population (1).

While the prevalences of knee OA estimated in the Framingham population were upwards of 30%, it should be noted that these data were based on the radiographic presence of disease. Upon evaluating the same population on the basis of symptomatic knee OA, the prevalence decreased dramatically. While 7.6% of individuals with no radiographic evidence of disease reported knee symptoms, only 19.2% of those with definitive osteophytes reported symptoms and 40.0% of those with definite disease reported knee symptoms (6;8). Females reported symptoms of knee OA more frequently than males with similar degrees of radiographic OA. It is evident, then, that the prevalence of knee OA appears lower when reporting symptomatic OA in those with radiographic OA because only a portion of those with radiographic evidence of disease actually

experience symptoms (1;6). In fact, the prevalence of patients with both radiographic and symptomatic OA have been estimated at <15% even among elderly populations. For example, only 6% of those > 30 years in the US have knee pain on most days compared to 10-15% of those age ≥60 years (13). It is generally thought that these figures are large underestimates of the actual disease prevalence. However, upon changing the definition of symptomatic OA to include any knee symptoms, even if they are not present on most days or many activities, the prevalence of knee symptoms increases towards 25% of the population (1). Likewise, it has also been shown that only approximately 12.5% of patients > 55 years of age who experience knee symptoms actually have radiographic OA suggesting that knee pain may be associated with many factors other than OA (1). Thus, the prevalence rates of knee OA differ significantly based on the diagnostic criteria implemented in the study and that radiographic OA is more prevalent than symptomatic OA in radiographically defined cases.

Few studies estimating incidence rates of knee OA exist, likely due to the fact that studies must be carried out over long durations in order to observe new cases, the methodological issues surrounding the definitions of OA and higher morbidity and mortality rates among the elderly who are most profoundly affected by OA. However, despite these challenges, some of the best incidence data have been derived from the Framingham

study which estimated the rate of incidence of knee OA. Over a mean interval of 8.1 years, a mean incidence of 2% per year for radiographic knee OA was revealed and an incidence of 1% per year was observed for symptomatic OA. The incident rate of knee OA in females was approximately 1.7 times that in males, data which is consistent with prevalence rates discussed previously (6;14).

Among different racial groups, the prevalence of OA varies. While the prevalence of knee OA in African Americans appears to be similar to Caucasians, African Americans seem to have more severe disease. A study conducted in China designed similarly to the Framingham study revealed prevalences of knee OA in the Chinese male population to be similar to those in American males from the Framingham study but estimated the prevalence in Chinese females to be significantly higher than American females (15). Knee OA affecting the lateral compartment was almost twice as common in the Chinese population compared to the Framingham population while the prevalence of medial compartment OA was similar in the two groups. A follow-up study suggested that racial differences in knee alignment and the frequency of squatting could explain differences between the populations and the higher prevalences of disease in females (15;16).

As evidenced by a plethora of epidemiological studies, it is apparent that knee OA affects all races worldwide. With an increasing incidence and prevalence of osteoarthritis associated with aging, it is expected that the extended life expectancy will result in greater numbers of people affected by OA. The burden felt by the healthcare system and by society as a whole will likely be greatest in countries that have seen increases in average life expectancy (17). For example, in the United States, it is projected that 59.4 million individuals will be affected by the disease by 2020, an increase of almost 50% from the 1995 estimate of 40 million individuals (10). Knee OA has long been recognized as a major cause of impaired mobility, especially among women. In fact, the disease was estimated to be the eighth leading non-fatal burden of disease in the world in 1990 with direct costs of medical intervention and indirect costs associated with premature mortality, chronic and short-term disability in western countries estimated at 1-2.5% of the gross domestic product (18;19).

As previously mentioned, the diagnosis of knee OA can be based on the symptomatic presentation of the individual (i.e. morning stiffness and joint pain), which is usually the case in patient-centred studies, the radiographic evidence of disease, as is typically used in community-based studies, or a combination of these (20). Disease progression is usually measured by changes in symptoms and/or changes in radiographic

features as presented on X-ray. A disease-specific instrument widely used to evaluate osteoarthritic symptoms is known as the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC™ Index). Initially developed in 1988, this measure evaluates patient-relevant symptoms and physical disability associated with hip and knee OA (21). Using this tool, changes in health status can be monitored and the effectiveness/efficacy of treatment interventions, pharmacological and non-pharmacological (i.e. acupuncture) can be measured. Since its initial validation, many additional studies have reported the validity and reliability of the WOMAC in various populations (22). In addition, the index has also been translated into many different languages (e.g. Spanish, Arabic and Italian) making the measure available for multi-center studies and making comparisons between populations possible (23-25).

Radiographically speaking, the disease pathology is reflected by two main features, those being a narrowing of the joint space, typically in the tibiofemoral compartment, and osteophyte formation. Diagnosis of the disease relies on the presence and/or severity of these features while progression is reflected by their worsening as indicated by increased joint space narrowing or an increase in the number or size of osteophytes. Until recently, however, semi-quantitative scoring systems (i.e. grading features on a scale of 0 to 3/4) such as the Kellgren-Lawrence scale specifically used for joint space narrowing and osteophytes have been

used to monitor these radiographic features. In fact, initially described in 1957, the Kellgren-Lawrence scoring system was accepted by the World Health Organization (WHO) in 1961 as the gold standard for cross-sectional and longitudinal epidemiological studies and has since been the classic source of measurement of these changes (26;27). Recently, however, problematic limitations of the scale have been noted including its insensitivity to change over time and the introduction of subjectivity in measurement leading to possible weaknesses in reproducibility.

These recent limitations have arisen as a consequence of supply and demand. Years ago, the treatment for knee OA was centred upon the management of disease symptoms using both pharmacological and non-pharmacological means. However, the magnitude and severity of the intensified burden associated with an increase in the prevalence of knee OA combined with disturbing and often overwhelming personal, social and healthcare costs, (i.e. increased morbidity, lost productivity, cost of treatments) has necessitated the development of disease-modifying rather than simply symptom-modifying agents. The demand for such agents exists and continues to grow while the supply of such agents seemingly lags behind. However, in order to demonstrate disease-modifying properties of pharmacological agents, it is essential to develop methods which can be used to measure aspects of the disease process. Thus, there has also been a great demand for the discovery of new techniques

which can measure outcomes of relevance to the pathophysiology of the disease. Because the so-called "supply" of techniques, such as the Kellgren-Lawrence scale and other semi-quantitative scoring systems, have not been deemed capable of doing so, other methods of assessing radiographs has become necessary.

To objectify the scoring of radiographs and improve the precision of measurement while evaluating a feature of the knee relevant to the disease, the notion of quantifying the amount of space between the distal femur and the proximal tibia, known as the joint space width (JSW), became the new tool used to measure disease progression. Because cartilage degeneration is the signature feature of knee OA, it was determined that these joint space measurements would be an indirect measure of the articular cartilage in the joint. Initially, radiographs were acquired in the supine position and JSW values were quantified by trained readers or radiologists using a magnifying lens. In time, this methodology has evolved to involve weight-bearing X-rays, which more accurately reflects the position of standing and visualizes the joint surfaces which come into contact while walking, and automated measurements of JSW using specially designed software algorithms. In fact, techniques for accurately and reproducibly acquiring radiographs and measuring JSW have improved significantly over the years.

Low cost and convenience have encouraged the use of conventional radiography in imaging OA and, today, it remains the primary imaging technique implemented for the diagnosis and longitudinal follow-up of patients affected by the disease (28). Unfortunately, this modality has several weaknesses due to the fundamental nature of plain radiography. For instance, X-rays permit the visualization of bone but not soft tissue structures known to be involved in the disease such as articular cartilage, menisci, synovium and ligaments. Thus, as previously noted, joint space measurements represent only an indirect measure of cartilage loss through the evaluation of the distance between two opposing articular surfaces. In addition, radiography represents only a two-dimensional picture of the joint and thus reveals joint space measurements at only one location between the joint surfaces (3;28). It is well known that cartilage loss is heterogeneous throughout the joint. Although the position of the tibia varies little with knee flexion, even slight bending of the knee can alter the corresponding point of articulation between the femur and the tibia. Thus, JSW measurements may be subject to positioning error which may complicate measures of longitudinal changes using repeated radiographs (3).

While plain radiography may be the simplest and least expensive method of imaging an osteoarthritic knee, advances in sophisticated imaging techniques since the mid-late 1990's have shown that magnetic

resonance imaging (MRI) may be the most promising in terms of advancing the current body of knowledge of OA and measuring disease progression with increased sensitivity. Its ability to provide multiplanar tomographic images of the knee eliminates the problem of reproducible positioning that arises in plain X-ray measurements of joint space and allows for the visualization of both bone and soft tissue features of the knee. In doing so, the knee can be evaluated as an organ rather than as different components analyzed individually. The direct visualization of articular cartilage allows for the quantification of various parameters of the tissue to be made. Advances in software and technology have led to the development of programs capable of measuring cartilage volume and thickness where the role of other tissues that may be included in joint space analyses on X-rays (i.e. meniscus) are of little concern (3;28). Unfortunately, however, disadvantages to using MR technology in osteoarthritis also exist, the primary one being access to scanning equipment. The limited number of machines available combined with patient wait times do not allow for clinical monitoring of disease progression. In addition, the cost of using such equipment in hospital settings and the need for physicians and technicians trained in MRI are also significant barriers. Thus, in Canada at the present time, the use of MRI is limited primarily to research in the effort to increase our knowledge and understanding of the complex disease process and identify other potential surrogate measures of disease progression.

In addition to MRI, some researchers have also begun to use Dual X-ray Absorptiometry (DXA). Typically used to analyze bone mineral density (BMD) in those with metabolic bone diseases such as osteopenia and osteoporosis, there has recently been interest in investigating measurements of BMD in those with OA. This has most likely occurred because of the hypothesis that bone, specifically subchondral bone, may play a significant role in the pathophysiology of OA (29-32). Thus, in addition to using MR imaging to look at subchondral bone, DXA allows for the quantification of bone density in various locations throughout the body including those regions adjacent to locations of joint disease. Because DXA equipment is more readily available and less expensive to purchase and operate than MRI, it is possible to investigate subchondral bone in those with OA to determine if, in fact, bone density is altered by the disease.

The increasing prevalence of knee OA, the increasing costs associated with the disease, both monetary and otherwise, and the demand for techniques that can reliably diagnose and monitor longitudinal disease progression warrants the investigation and quantification of disease parameters that can be measured using these imaging techniques. However, measurements of joint space width, cartilage volume and thickness and bone mineral density in osteoarthritic individuals mean little if there are no healthy reference values which can

be used as a comparison. Such is the case in osteoporosis where optimal "healthy" BMD measurements have been established such that comparisons can be made with those suspected of having the bone disease. Without these reference values, relative statements about "low" BMD would have no meaning. Thus, the purpose of this study was to quantify, in healthy males and females of ages 20-70 years, "normal" ranges of minimum JSW, cartilage volume and thickness BMD in the subchondral regions of the distal femur and proximal tibia. These values could then be used as reference points for comparisons with those diagnosed with knee OA. In addition, a second objective was to compare these values amongst one another to identify whether these variables were significantly related to one another in both healthy and osteoarthritic participants.

In establishing these objectives, many hypotheses were formed:

- JSW will decrease slightly yet consistently with aging in healthy and osteoarthritic males and females
- JSW measurements will be greater in males vs. females
- JSW measurements will be significantly smaller in those with knee OA compared to those without
- BMD in the subchondral regions of the tibia and femur will decrease with aging

- BMD in the subchondral regions of the tibia and femur will be greater in males than females and greater in those with OA compared to those without
- Patterns of cartilage volume and thickness will mimic those of JSW
- A significant, positive correlation will be identified between JSW and cartilage parameters in healthy and osteoarthritis individuals
- BMD measurements will correlate negatively with JSW in healthy individuals but this relationship will be much stronger and more pronounced in those with OA
- BMD measurements will correlate negatively with cartilage volume and thickness values in healthy individuals but this relationship will be much stronger and more pronounced in those with OA

Methodology

2.1 Subject Population

Four two groups of volunteers were recruited to undergo knee X-rays, DXA scans and knee MRIs. These groups included healthy men and women between 20 and 69 years of age, and osteoarthritic men and women over the age of 30 years. Healthy individuals were recruited via locally posted advertisements in hospitals, medical buildings and the athletic centre at McMaster University. Individuals were asked to respond to the advertisements by telephone. They were then asked a number of screening questions to ensure that they met the inclusion and exclusion criteria of the study. Those excluded from the healthy group (N=8) were individuals experiencing knee pain, those who had been previously diagnosed with a bone or joint disease (i.e. rheumatoid arthritis, osteoporosis) and those who had sustained a knee injury. Participants with knee osteoarthritis (OA) were referred by local rheumatologists or were respondents to locally posted advertisements. In the latter case, these individuals claimed to have been diagnosed with knee OA by their physician. Osteoarthritic individuals excluded from the study (N=6) included those with diabetes or a bone or joint disease other than OA (i.e. osteopenia, osteoporosis, RA) and those who had previously undergone knee surgery (i.e. arthroscopic surgery). All participants were informed, both verbally and in writing, about the three scanning procedures involved

in the study, namely a knee X-ray, bone mineral density scan and an MRI of the knee, and provided with information on the measures involved. Individuals who were eligible to have a knee X-ray and a bone mineral density scan but were not eligible to have a MRI scan of the knee due to contraindications such as implanted devices (i.e., pacemaker) or vascular implants (i.e. stent, aneurism clips) agreed to undergo those two scans. All participants were required to sign a consent form that had been approved by the Research Ethics Board at St. Joseph's Healthcare. In addition to completing the study consent form, individuals were required to complete two questionnaires, one of which was an MRI safety form and the other which asked questions pertaining to his/her medical history, medications and exercise activity. Osteoarthritic participants were asked to complete the WOMAC™ Index and the SF 36 health survey (21;33;34).

The entire population under investigation consisted of 179 individuals, 124 of whom were "healthy" and 65 of whom were diagnosed with knee OA. The vast majority of participants were Caucasian (approx. 92%) and non-smokers (approx. 92%). Of those who affirmed they were current smokers, three quarters of them stated they had smoked cigarettes for ≥ 5 years. Less than 5% of participants reported taking non steroidal anti-inflammatory medications, corticosteroids and thyroid or parathyroid hormone on a regular basis at the time of the study. Surprisingly, 3 osteoarthritic individuals reported taking glucosamine

sulfate and/or methylsulfonylmethane (MSM) while, surprisingly, so did 3 healthy individuals. Of the females who participated, approximately one fifth of them reported taking hormone replacement for menopause or as a method of birth control. Approximately 40% of participants reported that they participate in "regular" physical activity. More specifically, 44% of healthy individuals reported being physically active compared to only 22% of those with knee OA, likely due to the physical limitations presented by the disease.

Study volunteers underwent all three scans during a single two hour clinic visit. Participants were permitted to withdraw at any point in the study without bias. A description of the three scanning protocols follows.

2.2 Plain Radiography

Conventional radiography has been, and continues to be, the primary imaging modality used in the evaluation of osteoarthritis (OA), both in terms of diagnosis and monitoring of disease progression. Unfortunately, this imaging technique presents numerous limitations including its inability to directly visualize non-osseous tissues known to be involved in the pathophysiology of OA, most notably articular cartilage, menisci and synovium, and its capability of imaging only in two-dimensions (28;35). Despite these limitations, plain radiography also presents many advantages. Abnormalities such as osteophytes,

subchondral sclerosis and cysts can be detected on plain film. In addition, cartilage degeneration in OA can be indirectly evaluated by measuring the space between opposing articular surfaces, known as joint space width (JSW). By comparing baseline radiographs with those taken at later time intervals, it is possible to objectively assess disease progression by comparing the number and size of osteophytes and the rate of narrowing of the joint space width.

For decades, the semi-quantitative scoring system known as the Kellgren-Lawrence (K-L) scale was accepted as the standard for the longitudinal evaluation of knee OA (35;36). Unfortunately, the ambiguity of the definitions of each grade on the scale, the low sensitivity to change, the element of subjectivity introduced by the nature of grading and the focus on the presence of osteophytes have challenged the use of this scale in research and in clinical practice. Definitions of each K-L grade are displayed in Table 1. More objective, sensitive methods for evaluating knee OA have since been introduced, the most popular of which is the automated measurement of JSW. In fact, guidelines endorsed by OMERACT (Outcome Measures in Arthritis Clinical Trials) and the FDA suggest that JSW be considered one of the primary outcome measures in trials of osteoarthritic agents longer than one year in duration (37-39).

To date, many groups have developed methods of acquiring and analyzing radiographs for the purpose of measuring JSW. In terms of acquiring a knee X-ray, different imaging protocols vary in terms of joint positioning and radiographic procedures as shown in Table 2 and Figure 3 (28;40-42). For instance, the degree of knee flexion and foot rotation, the angle of inclination of the X-ray beam and the degree of magnification have all been adjusted to optimize both the quality of the X-ray and the reproducibility of the technique. Limiting or even eliminating causes of measurement error in the positioning technique is critical as there are many sources of variability which can be introduced at different points in the acquisition process. For instance, the quality of medial tibial plateau alignment and the degree of knee flexion and rotation have been reported as potential significant sources of error (35;43;44). Ideally, the goal of all techniques is to limit the sources of variability of patient positioning, optimize the sensitivity of measurement and achieve the highest possible reproducibility of measurement such that any changes in JSW between successive time periods can be attributed to actual changes at the joint and not to measurement error. Although numerous comparisons of different techniques have been published, to date no single technique has been deemed "the gold standard".

In addition, some techniques including the semi-flexed and Lyon-Schuss techniques make use of fluoroscopy while the metatarsal-

phalangeal (MTP) and the fixed-flexion techniques do not. While fluoroscopically guided radiographs have been shown to provide good precision in multi-centre studies, these techniques are costly to employ due to the required presence of a radiologist and expose patients to doses of radiation much higher than techniques that are non-fluoroscopic (45;46). In addition, it has been shown that the test-retest reproducibility of non-fluoroscopically guided X-rays may be as good as fluoroscopically guided X-rays acquired using the fixed-flexion technique (root-mean-square standard deviation = 0.1 mm) (42). Also, it has been shown that the reproducibility of measurement of minimum joint space width (mJSW) is better when using an automated computer algorithm compared to manual methods (i.e. hand-held lens) (42).

Based on observations and published data, the fixed-flexion technique has been shown to be reproducible due, in part, to its ability to decrease possible sources of measurement error introduced by fixing the degree of femoral and tibial angulation (flexion and rotation). It is more cost-effective than fluoroscopically assisted techniques which have limited multi-centre applications and exposes those who are undergoing X-ray to the smaller doses of radiation. In addition, automated algorithms used to quantify mJSW are more accurate and reproducible than manual analyses using magnifying lenses. It is for these reasons that the fixed-flexion technique was selected as the positioning technique of choice and the

automated computer algorithm was selected to accurately measure mJSW.

Each participant underwent a single fixed-flexion knee X-ray of his/her non-dominant knee. In this position, participants are required to stand, with their weight distributed equally between their legs, on a piece of cardboard such that both great toes are touching the vertical X-ray table and feet are externally rotated by approximately 10°. In this position, both feet are traced onto the cardboard should the foot map be needed for use in successive X-rays. Facing the vertical X-ray table and holding the sides for balance and support, subjects are asked to bend their knees slightly such that both their patellas and thighs are pressed tightly against the table. In doing so, the position of the femur and tibia are fixed, and thus, so is the degree of knee flexion. The posteroanterior X-ray beam is directed parallel to the tibial plateau (10° caudal beam alignment). A schematic representation of the fixed-flexion technique is shown in Figure 3 (right hand side).

Radiographs were graded independently by two radiologists according to the Kellgren-Lawrence (K-L) scoring system. In the case where the radiologists did not agree on the K-L grade assigned to an X-ray, the X-ray was viewed by both radiologists simultaneously and a consensus grade was assigned. This grade was used to confirm or refute

the radiological presence of knee OA and to assess the degree of disease severity. Those assigned a grade of 0 or 1 on the scale were considered to be "healthy" while those scoring ≥ 2 were considered to have knee osteoarthritis.

X-rays were digitized using a Sierra plus™ digitizer (Vidar Systems Corporation, Herndon, VA, USA) at an isotropic pitch of 84.7 μm and a 12 bit grey scale resolution. The digitized images were further analyzed for mJSW in the medial compartment of the knee using an automated computer algorithm, details of which have been described previously (47). An analyzed radiograph is depicted in Figure 4. This program delineates the bony margins of the femoral condyles and the tibial plateau. Radiographs were successfully analyzed except in the cases of severe arthritis (i.e. cases of K-L grade 4 radiographs). In these instances, the computer was unable to detect the margins of the femoral condyles due to the severity of the disease with resultant bone on bone contact of the medial femoral condyle and the tibial plateau. In this case the mJSW was assigned a value of 0 mm. In approximately 3% of radiographs analyzed for mJSW, user intervention was required to slightly alter the delineations drawn by the computer algorithm. All manual manipulations were considered minor and did not alter the actual mJSW values in a significant way. These data are represented in the Results section of this thesis.

Each participant's mJSW values were then entered into a large patient database.

2.3 Dual X-ray Absorptiometry

Currently, dual energy X-ray absorptiometry (DXA) technology remains the most widely used technique for the measurement of bone mineral density (BMD). Exposing patients to low doses of ionizing radiation (approximately 2 μSv per scan, equivalent to less than the exposure to natural radiation and radioactivity that every Canadian receives in one day), this non-invasive technique is a fast, accurate and reproducible method of assessing bone mineral density (48;49). Typically, BMD measurements are used to assess bone loss associated with aging, to aid in the diagnosis and longitudinal follow-up of those with osteoporosis, to assess potential fracture risk and to evaluate the effectiveness of various intervention strategies including pharmaceutical agents and exercise (50-54). Clinically, BMD measurements are made typically at the lumbar spine, proximal femur (hip) and the forearm (distal radius).

The distal femur and proximal tibial regions are not commonly scanned using DXA technology. In fact, BMD measurements in these areas have never been used in clinical practice to study metabolic bone diseases. However, decades of research in the area of OA have

determined there is little doubt that subchondral bone plays a significant role in the pathogenesis of knee OA. In fact, over 30 years ago it was suggested that articular cartilage was protected from damage by subchondral bone shock absorption which occurred during impact loading and that increased mechanical stress on cartilage may have been caused, in part, by a high periarticular bone mass (55;56). In the late 1980's, researchers began to use DXA technology to study subchondral bone, initially in the proximal tibia and later also in the distal femur, to try to elucidate more information about its role in the onset and progression of OA (50;57).

To date, different groups studying subchondral bone density have published results determining BMD primarily in regions of the proximal tibia, although distal femoral BMD has been reported. However, there is no standardized method of evaluating BMD in either of these areas. Different studies have employed various techniques of measurement making comparisons of data between these studies a challenge (48;50;57-59). Listed below are ways in which these methods differed:

Patient positioning:

In one study, patients were asked to lie in a supine position with the knee flexed at 30° (58) while in others, patients sat upright or lay supine with knees fully extended (50;57;59;60). One study did not mention

whether or not the knees were flexed (48). While most studies commented on "stabilizing" or "fastening" the foot, no study commented on whether or not the foot was rotated.

Number and Size of Region(s) of Interest:

There has also been great variation in the number and size of the regions of interest in the proximal tibia in studies that have been published. In one study, a 2.01 cm high region running the entire width of the bone was chosen as the region of interest in the proximal tibia (50) while a 5.6 mm high region of 1.0 cm in length was selected in another study (60) and a single square with an area of 1 cm^2 was used as the region of interest in the medial part of the proximal tibia in yet another study (57). In contrast, one study "selected" seven regions of interest of various sizes and orientations ranging in area from 0.09 to 0.23 cm^2 in the proximal tibia (58). Seven regions of interest of 1 mm in height and the entire width of the proximal tibia were the areas investigated in the study by Murphy et al. (48) while three regions of interest in each of the medial and lateral compartments of the proximal tibia were investigated by Hulet et al. (59). Most studies reported precision measurements in the area of 1.89-5.30%. However, it is important to note, here, that each technique used to quantify subchondral BMD in the proximal tibia used a region of

interest of a fixed size as depicted in Figure 5. In doing so, one issue that arises is that of differing bone sizes based on patients of different sizes. For instance, measuring 1 cm^2 of bone in a patient with a large tibia may include only a small area of trabecular bone while measuring this same area in a patient with a small tibia may mean scanning a much larger, proportionally speaking, region of interest. Likewise, quantifying BMD in a region of interest which is 5.6 mm or 2.01 cm in height may lead to inconsistent results between patients based on differences in bone size. One way in which this issue can be avoided is by investigating regions of interest of a fixed proportion of bone rather than a fixed area or size of bone. Evidently there was no consistency between studies with respect to the number or sizes of the regions of interest under investigation. In fact, the lack of detail reported in these studies was surprising and it appeared, at times, as though the number and sizes of regions were chosen arbitrarily without justification or explanation. In addition, one must remember that results of these studies are presented as areal (2D) BMD and not volumetric (3D) BMD. As a 2-D measure of a 3-D structure, areal BMD may give falsely elevated results because of bigger bone size.

Location of Region(s) of Interest:

While all studies expressed BMD measurements in the subchondral regions of the proximal tibia, the exact locations of the regions of interest varied greatly between methodologies. The study by Bruyere et al.

investigated regions with the highest degree of sclerosis at the narrowest joint space in the medial compartment of the knee while other studies used the fibula and the intercondylar notch as references point to mark the starting point for the height of the measurements (48;59). This is also shown in Figure 5. Again, it was evident that there was no consistency between scanning techniques particularly in relation to the location of the regions of interest being studied, that regions appeared to be selected arbitrarily and justifications for investigating these regions of interest were weak, if existent at all.

The technique for scanning BMD implemented in this study was developed and validated in a population of individuals with spinal cord injuries in 2001 (61). This protocol was designed with a number of specific important features in mind. Firstly, the knee was slightly flexed so as to ensure adequate separation between the distal femur and the proximal tibia such that BMD results of the regions of interest in the subchondral regions directly adjacent to the articular cartilage would not interfere with one another. Secondly, the foot was internally rotated for the purpose of maximizing the amount of space between the tibia and the fibula while still being able to view the entire tibia in the posteroanterior direction (not laterally). This was also necessary for minimizing the amount of overlap of the fibula with the tibia such that the analysis of the proximal tibia included as little proximal fibula as possible (seen in region

T2 of Figure 9). Lastly, this protocol selected regions of interest in both the proximal tibia and the distal femur based on the length of the entire bone being scanned. For instance, each region of interest includes a well defined percentage of the entire bone length so as to make BMD measurements in different patients comparable with one another. Compared to previously published protocols which defined regions of interest by area or height, this technique defined regions of interest by approximating the length of the bone from the width of the epiphysis and subsequently separating the distal 25% of the femur and the proximal 25% of the tibia each into four regions of interest also based on proportions of the length of the bone. The advantage of doing this allows a standardization of measurement and permits data from patients of all sizes to be compared with one another based on these defined areas.

Each participant agreed to undergo DXA scans of his/her lumbar spine and non-dominant hip acquired using a Hologic Delphi™ DXA scanner (Hologic Inc., Bedford, MA, USA). Bone density analyses were performed by a trained technologist using the scanner's own software. This same scanner was used to acquire scans of the distal femur and proximal tibia for each individual. Subjects were required to lay, supine, on the table, with the non-dominant leg held in place by a polycarbonate positioning device (Figure 6). Using this device, knee flexion of 5° was achieved by placing a curved polycarbonate insert behind the knee while

the foot was placed in a foot plate and internally rotated by approximately 10°. This position has been found to optimize both the space between the femur and the tibia as well as the space between the fibula and the tibia (61). For the purpose of imaging the distal femur, the laser crosshair of the densitometer was positioned 5 cm distally from the inferior border of the patella where the scanning began and proceeded 24 cm proximally (Figure 7). Before starting the proximal tibia scan, the laser crosshair was positioned 23 cm distally to the superior border of the patella where the scan began and again, proceeded proximally 24 cm. The femur and tibia were positioned such that the shaft of the bone appeared as vertically as possible in the DXA scan and that the epiphysis was centred in the image. Distal femur and proximal tibia scans were acquired using the lumbar spine scanning software associated with the Delphi machine. The distal femur and proximal tibia DXA scans were analyzed using the protocol as described elsewhere (61). Each of the femur and tibia are divided into 4 distinct regions, F1-F4 and T1-T4, respectively, where F1 and T1 are the most proximal and F4 and T4 are most distal (Figures 8 and 9). For the distal femur, the following is a breakdown of the sizes of each region of interest: F1 accounts for 5.1% of the entire bone length, F2 accounts for 7.7%, F3 for 10.0% and F4 for 2.6%. Likewise, the proximal tibia is divided into four regions in a similar fashion where T1 accounts for 5.1% of the bone length, T2 for 5.1%, T3 for 11.7% and T4 for 8.3%. Average distal femur and proximal tibia BMD measurements were also calculated

using the software. Scans were saved and archived by technicians at the end of each day and analyses of BMD in the femur and tibia were conducted only after approximately 30-40 scans had been acquired. All data was entered into patient database where it was later analyzed.

2.4 Magnetic Resonance Imaging

Despite significant advances made in techniques used to both image and analyze plain radiographs in past decades, magnetic resonance imaging (MRI) has emerged as the imaging technique most likely to expand and improve our knowledge of OA pathophysiology and provide us with information that may aid in the evaluation of disease-modifying therapeutic agents. Because plain radiography is limited by its inability to visualize tissue other than bone and acquire images in three dimensions, mJSW represent only a surrogate measure of cartilage thickness, can only be measured in the tibiofemoral compartment of the knee and is susceptible to error caused by variability in patient positioning (28;62). In contrast, MRI has an unparalleled ability to visualize soft tissue detail in three dimensions enabling us to both qualitatively and quantitatively evaluate structures involved in the disease. In addition, the ability of MRI to produce multiplanar tomographic images eliminates problems which exist with plain radiography such as the superimposition of overlapping tissues and the potential for error introduced by patient

positioning making MRI ideally suited to imaging synovial joints (28). For these reasons, MRI has been used extensively in the study of knee OA with a specific focus on articular cartilage morphology.

Since the late 1990's, numerous research groups have worked to determine what they consider to be the "optimal" MRI pulse sequence for imaging articular cartilage. These efforts have tried to obtain the highest possible spatial resolution and signal-to-noise and contrast-to-noise ratios while trying to preserve a minimal acquisition time. The goal of doing so is to optimize contrast at the bone-cartilage interface for delineating the joint surface essential to achieve the highest possible accuracy and precision of measurement. Although a 1.5 mm slice thickness and a 0.3 mm in-plane resolution have commonly been used, there remains a lack of consensus as to which scanning protocol is the "best" (62).

A recent paper published by Eckstein and Glaser reviewed MR scanning protocols that have been used to image cartilage over the last ten years (62). While the techniques of fast spin gradient-echo, selective water excitation and others have been reported to produce images of comparable quality and shorter scan times to the fat-suppressed, T1-weighted, three-dimensional (3-D) spoiled gradient echo (SPGR), the latter has been the most commonly used protocol (28;62-65). Although this sequence provides superior signal and contrast for the delineation of

the bone-cartilage interface and the articular cartilage surface, its primary disadvantage is the relatively long scan time (typically ≥12 minutes using a 1.5 Tesla MRI) which often contributes to decreased image quality due to motion artifact (28).

While the discovery of an optimal scanning protocol is extremely important in the effort to quantify tibial and femoral cartilage volume and thickness, image acquisition represents only a part of the procedure. The other challenge is the implementation of a software program with the ability to measure with accuracy and precision the variables with minimal user intervention in the shortest possible time period. Various cartilage segmentation algorithms have been developed, each requiring a degree of user intervention, in some cases an extensive degree, and quality control by an experienced operator (62). These algorithms include the manual tracing of the borders of the articular cartilage while others use what is referred to as "region growing" and "B-spline snake" techniques as reviewed by Eckstein and Glaser (62). Ideally, algorithms considered to be semiautomated should require less time and user-intervention (thus improving intra and inter-rater reliability) than methods that are manual (66).

With few exceptions, articular cartilage volume and thickness measurements yielded by these segmentation algorithms have been validated by comparing values obtained through MR image analyses with

those obtained from computed tomography arthrography or the actual quantification of cartilage volume and thickness of surgically removed tissue from human and animal cadaveric specimens (62;64;67-69). Accuracy and reproducibility of these various segmentation algorithms have also been investigated and reviewed (62). For scans acquired in the sagittal plane with a slice thickness of 1.5 mm, accuracy and reproducibility data from previously published studies are presented in Tables 3 and 4, respectively.

For the purposes of this study, MR scans were acquired using a FDA approved 1.0 Tesla pMRI system (OrthOne™, ONI Inc., Wilmington, MA, USA), approved for use in Canada by the Therapeutic Products Directorate of the Canadian Government (Figure 10). Subjects were seated in the scanning chair with their non-dominant knee fully extended and centred within the iso-centre of the 180 mm removable quadrature volume transmit-receive coil. Padding was placed around the knee and leg to decrease the potential for movement. In the majority of cases, patients chose to recline back in the chair and place the non-scanning foot onto a step-stool to relieve pressure on the lower back. All study participants were positioned and scanned by one experienced operator.

To ensure the scanning knee was correctly positioned, a sagittal entry locator scan with a 2-D gradient-echo sequence and an axial entry locator scan with fast spin echo were performed (scan time 2-3 minutes).

Following this, a 3-D SPGR scan was acquired in the sagittal plane using the following parameters: TR 60 ms; TE 12.4 ms (or minimum); flip angle 40°; bandwidth 30 kHz; matrix 512 x 256 (frequency x phase); 1 excitation; field of view 150 mm; slice thickness 1.5 mm; 56 to 64 partitions depending on patient size; fat saturation (FS) and radiofrequency spoiling; scan time 15-16 minutes. Acquired scans were then transferred to an independent workstation where they were saved in DICOM format and later deleted from the scanning computer. Upon completing the acquisition of images from study participants, images were saved on a portable hard drive and transported to an image analysis centre at the Paracelsus Medizinische Privatuniversität in Salzburg, Austria. One person, trained in the use of the proprietary segmentation software (Chondrometrics GmbH, Ainring, Germany), was responsible for analyzing all images. Cartilage segmentation was conducted on a slice-by-slice basis by manually tracing the bone-cartilage interface and the cartilage surface of the medial tibial plateau (MT) (66;70). After segmenting all images, the same reader reviewed the segmentation of all images for the purposes of quality assurance and alterations in segmentation were made if deemed necessary. Medial tibial cartilage volume and thickness measurements were then computed and values were entered into the patient database.

To determine if, in fact, measurements of cartilage volume and thickness are reliable, an additional reader agreed to analyze a small number of MR images. Blinded to the measurements of the first reader, raw images without any analyses from the first reader were analyzed independently by a second trained reader. Comparisons between measurements of medial tibial cartilage volume and thickness were made and are reported in Chapter 5.

In addition to assessing tibial cartilage volume and thickness, MR images from a sub-group of healthy individuals were analyzed, using a semiquantitative scoring system developed in-house, for the presence and severity of other bony and soft-tissue abnormalities. The purpose of doing so was to estimate the prevalence of such abnormalities, namely subchondral cysts, bone marrow edema, cartilaginous lesions and meniscal and ligamentous defects, in an asymptomatic, seemingly healthy population. A similar scoring system used to evaluate bony and soft tissue features of the knee was developed by Peterfy et al. This system is termed WORMS, a pneumonic for Whole Organ Magnetic Resonance Imaging Score (WORMS) (71). More recently, an additional scoring system, termed the Knee Osteoarthritis Scoring System (KOSS), which assessed the same features of OA as those assessed in our scoring system, has also been developed (72). Once again, results of this sub-study are presented in Chapter 5.

Chapter 3: Minimum Joint Space Width Results

In total, 120 healthy individuals without a history of knee pain, injury and a bone or joint disease agreed to participate in the X-ray portion of the study. Of these, 73 were female and 47 were male. The mean (standard deviation (SD)) height, weight and body mass index (BMI) of the healthy female group was 1.65 (0.06) metres, 66.5 (14.0) kilograms and 24.7 (4.9) kg/m^2, respectively. For the group of healthy males, the mean (SD) height, weight and body mass index (BMI) was 1.77 (0.07) metres, 80.7 (12.8) kilograms and 25.6 (3.7) kg/m^2, respectively. K-L grading of X-rays revealed that, of the healthy women, 49 had radiographic scores of 0 while the remaining 24 had scores of 1. Grading for males revealed that 31 had K-L scores of 0 and 16 had a score of 1.

Thirty-three osteoarthritic women participated with a group mean (SD) height, weight and BMI of 1.58 (0.07) metres, 72.4 (12.9) kilograms and 28.1 (5.4) kg/m^2, respectively. The group of osteoarthritic men included 22 individuals with 1.81 (0.07) metres, 88.8 (14.5) kilograms and 27.8 (3.7) kg/m^2 being the group mean (SD) height, weight and body mass index (BMI), respectively. Of the 33 female participants, K-L grading revealed the following; 3 grade 0, 8 grade 1, 12 grade 2, 5 grade 3 and 5 grade 4. Thus, although all of these participants had been clinically diagnosed with knee OA, only two thirds of them (67%) showed radiographic evidence of the disease (K-L ≥ 2). K-L scoring of the

radiographs from the 22 males revealed 2 were grade 0, 2 grade 1, 9 grade 2, 3 grade 3 and 6 grade 4. Once again there were individuals who had been clinically diagnosed with knee OA but had no radiographic evidence of disease, although this proportion was much smaller at only 18%.

All individuals agreed to undergo a fixed-flexion knee X-ray as per the protocol described previously. However, in order to perform analyses to evaluate "normal" and osteoarthritic measurements of mJSW in these individuals, an analysis of the short-term and long-term reproducibility of the technique was undertaken. This was necessary in order to support the notion that this methodology was, indeed, a technique that could provide reproducible measurements of mJSW in this population.

3.1 Minimum Joint Space Width Reproducibility

A sub-group of subjects agreed to volunteer for a study conducted to assess the short-term and long-term reproducibility of medial mJSW measurements. The short-term reproducibility included 34 volunteers comprised of 7 men and 27 women. Nineteen of these volunteers had one knee radiographed twice while the remaining fifteen volunteers agreed to have both knees X-rayed under the fixed-flexion protocol, thus yielding 49 pairs of radiographs. After undergoing the initial X-ray,

volunteers returned to their seats in the waiting room and were then called back into the exam room for their second radiograph a short time later (approximately 15 minutes). Each radiograph was graded for OA severity using the K-L scale. Thirty-four pairs were graded as normal (KL grade 0 or 1) while 13 pairs showed radiographic evidence of OA (KL grade 2 or 3). Two pairs of radiographs scoring 4 on the K-L scale, reflecting bone on bone contact, were excluded from the analyses. For the long-term reproducibility study, a single knee radiograph from 11 females and 3 males was initially acquired. All individuals returned for their second X-ray between 8 and 26 months later. These radiographs included 10 of grade 0 and 4 of grade 1.

Each X-ray was initially analyzed for mJSW using the automated algorithm previously discussed (47). Subsequently, two readers (Karen Beattie and Pauline Boulos) independently reviewed each of the images and the computer-determined borders of the medial tibial plateau and the medial femoral condyle. In an attempt to optimize these pre-determined contours, reader 1 altered the computer-defined borders on 2 radiographic images in the short-term study and 3 images in the long-term study while the second reader made alterations on 4 short-term and 4 long-term radiographs.

Three measures of reproducibility were calculated from this data. The root-mean-square standard deviation (RMSSD) was used to assess the reproducibility error of repeat radiographs, the coefficient of variation (CV) examined the reproducibility error as a relative term and the intra-class correlation (ICC) coefficients measured reliability (47;73). Intra-class correlation coefficients, by consensus, are considered good when $\geq$ 0.80. Methods of calculating RMSSD and CV are shown in Figure 11. When including all participants in the analyses for the short-term study, the RMSSD was found to be 0.19 mm for the computer algorithm and 0.20 mm and 0.25 mm for each of the two readers, respectively. The short-term data were then re-analyzed after separating those with no radiographic evidence of knee OA from those with radiographic OA (i.e., K-L grades 0 and 1 together, K-L grades 2 and 3 together). In so doing, it was seen that the RMSSD for the "healthy" volunteers was improved over that for all participants, and that the reproducibility for the osteoarthritic individuals was, indeed, worse. The ICCs for the computer, when investigating all individuals as well as those who had and did not have knee OA, were 0.98, 0.98 and 0.97, respectively. For each reader, the ICCs for all individuals and for just those who had K-L scores of 0 and 1 were between 0.96 and 0.98, respectively. The RMSSD of the long-term study was found to be 0.14 mm for the computer algorithm and 0.17 mm for each reader. In addition, the ICC for the long term study was 0.99 for the computer and 0.98 for each reader, suggesting that manually altering

the bony borders determined by the computer algorithm did not improve the reproducibility in either study. In fact, by introducing subjectivity to the analyses, reproducibility actually decreased. These results and the corresponding CVs are shown in Table 5.

3.2 Quantification of Minimum Joint Space Width in Healthy Volunteers

Upon determining that the short-term and long-term reproducibility of the automated technique of analyzing mJSW were satisfactory, further analyses of mJSW in healthy individuals were conducted. Both groups, males and females, were each subdivided by decade of life, thus treating age as a categorical rather than a continuous variable. The purpose of doing so was to determine if there was an identifiable decade where changes (i.e. decreases) in mJSW could be detected. In addition, previous groups had performed analyses by age group and, by doing so in these analyses, comparisons between studies could be made possible. The mean (SD), range, minimum and maximum mJSW data for each of these age groups were calculated and are presented in Table 6. A graphical representation of the distribution of mean mJSW data per decade for both males and females is shown in Figure 12. Neither the descriptive statistics nor the graphs appear to show any differences in mean mJSW values between decades in either males or females. In fact,

it appears as though a "normal" value of mJSW for healthy women lies in the range of 4.6-5.1 mm while in healthy men, this range is 5.3-5.8 mm. Indeed, this was supported by results from an ANOVA analysis where there no significant differences in mJSW between age groups were identified even after considering BMI as a covariate ($p>0.05$). The only significant difference identified was that between genders. An ANOVA analysis performed with BMI, age and gender considered covariates revealed that men have significantly larger mJSW values than women ($p<0.05$).

In considering age as a continuous variable, without dividing the healthy groups of men and women into decades, the data were once again analyzed. A graphical representation of mJSW as a function of age for both healthy males and females is depicted in Figure 13. As shown in both Figure 12 and Figure 13, males indeed have significantly larger mJSW values compared to women of the same age or age group. Backwards linear regression analyses were performed to determine whether age was significantly related to mJSW in healthy individuals. These results determined that neither age nor BMI are significantly related to mJSW in either males or females ($p>0.05$). The slopes of the regression lines (β coefficients) (Figure 13) were -0.077 for males and -0.164 for females with 95% confidence interval lines also. These data

suggest that less than 3% of the variation in mJSW is explained by the variation in age in this population.

While there are no mJSW outliers existing in the healthy male population, it is evident from the graph of mJSW distribution in healthy women that two outliers exist, as depicted in Figure 13. These two points represent a 42 year old with an mJSW of 6.519 and a 57 year old with an mJSW of 7.341 mm. Upon removing these outliers from the data set, regression analyses were repeated and results revealed that age was, indeed, significantly related to mJSW. The slope of the regression line (red) was -0.284 ($p<0.05$) indicating that increasing age correlates with decreasing mJSW (Figure 14). It is very important to note, however, that these points were removed solely because they were outliers and there is no scientific justification or explanation for doing so.

3.3 Quantification of Minimum Joint Space Width in Osteoarthritic Volunteers

Just as in healthy individuals, mJSW analyses in osteoarthritic participants were also conducted. Both the male and female groups of participants were subdivided by age group and descriptive and graphical analyses were performed. Table 7 displays descriptive statistics of mJSW per age group while Figure 15 represents mean mJSW data in the form of

a bar graph. It is important to note that there were very large standard deviations and broad ranges in mJSW for many of the age groups in both the males and females. For instance, mJSW values ranged from 0 to just over 6.0 mm in both 50-59 and 60-69 year old females as well as 50-59 year old males. The variation in mJSW likely reflects the fact that there are small numbers of individuals in some of these groups and, within these age groups, there is a wide range of disease severity as reflected by K-L scoring. Given this observation, it was intuitive to investigate the distribution of mJSW as it varied with K-L grading in both males and females. As depicted in Figure 16, it appears as though mJSW decreases with increasing K-L score of ≥2, a grade which is defined by the presence of joint space narrowing observed on plain radiographs (Table 1). Inconsistent mean mJSW results represented by the bar heights for K-L grades 0 and 1 are also representative of the definitions of the scoring system as neither grade includes any comment of evidence of joint space narrowing. In both graphs in Figure 16, the mean mJSW values in grade 2 lie between grades 0 and 1 as in the male population, or are greater than those of both grades 0 and 1 as is true for the female population and also the study population as a whole. This disparity is likely the result of two factors, the first being the fact that the K-L grading system primarily focuses on a definition of knee OA based on the presence and severity of osteophytes, not JSW. The second is the use of the phrase "joint space narrowing" with the term "narrowing" being a relative, qualitative term as

opposed to mJSW which is an objective, quantifiable value. The issue arises of where the comparison of width should occur; Joint space narrowing in the medial compartment relative to the lateral compartment or relative to the medial compartment of the contralateral knee. The conflict in relating these two variables arises from the possibility of having a small, uniform joint space width (mJSW) that is not necessarily narrowed or, likewise, a larger joint space width which is narrowed. In this way, the term joint space narrowing would not necessarily relate to the actual joint space width. Ultimately, one would expect the mean values of mJSW to decrease consistently with increasing disease severity, and this was the case for mild, moderate and severe disease. The lack of consistency between K-L grades of 0, 1 and 2 is likely explained by the lack of an objective, quantifiable measure of joint space width or narrowing and the scoring system's predominant focus on osteophytosis.

In addition to exploring the relationship between disease severity as defined by the K-L scale and its quantitative counterpart, mJSW, the relationship between age and mJSW was also investigated. In females, mJSW appeared to decrease significantly with increasing age group and this was shown to be true when an ANOVA analysis was performed ($p<0.05$). Unfortunately, post-hoc analyses could not be performed due to the fact that there was only one data point in the 30-39 year old age group. The same analysis was performed in men and again, a significant

difference in mean mJSW was also identified in males of different age groups. However, a Bonferroni post-hoc analysis revealed that the significant difference in mJSW occurred only between the older two groups, 60-69 and 70-79 years of age ($p<0.05$). The variability in mJSW in the osteoarthritic male population, as shown by the relatively large standard deviations, is likely due, at least in part, to the small numbers of individuals in each age group. This is also true for the female groups. It is not surprising that it was difficult to recruit those under the age of 50 years who had been clinically diagnosed with knee osteoarthritis. Likewise, those in the older age groups (i.e., ≥ 70 years) are also difficult to recruit because of generally decreased states of health and the presence of comorbidities.

Minimum JSW measurements as they relate to age as a continuous variable were also analyzed in the osteoarthritic population. Figure 17 graphically represents the distribution of mJSW as it varies with age. In examining these graphs it is obvious that there is a very wide range in mJSW values in males and females of similar age. It is important to consider that there are varying degrees of severity between these individuals, ranging from "normal" to bone on bone contact, and this may help to explain the distribution and wide confidence intervals. In addition, one must remember that not all of these individuals showed radiographic evidence of disease as supported by the presence of K-L grades 0 and 1

X-rays in the osteoarthritic population. Thus, a proportion of these individuals had been diagnosed clinically, but not radiographically, and this may explain the presence of individuals with comparatively large mJSW values (i.e., mJSW > 6.0 mm), within the "normal range" as observed in healthy individuals in this population.

In considering the data together (i.e., male and female), a backwards linear regression analysis was initially performed to determine if gender was significantly related to mJSW. No significant differences were found between mJSW values in osteoarthritic men compared to osteoarthritic women. After separating the two sexes, backwards linear regression analyses were again performed to determine if age and BMI were significantly related to mJSW. In women with knee OA, both variables were significantly related to the dependent variable of interest with a standardized β coefficient of -0.479 for age and -0.349 for BMI ($p<0.05$). These results indicate that both increasing age and higher BMI are associated with less space between the femur and tibia. In men with knee OA, only age was found to be negatively associated with mJSW (β coefficient of -0.431 ($p<0.05$)).

3.4 Relationships between Minimum Joint Space Width and Pain and Function in Osteoarthritic Volunteers

The WOMAC and SF-36 questionnaires were used to evaluate levels of pain and function in osteoarthritic volunteers. While the WOMAC assesses 5 related pain actions, 2 stiffness items and 17 functional activities related to OA, the SF-36 measures overall health status through assessing eight health concepts: 1) limitations in physical activities because of health problems; 2) limitations in social activities because of physical or emotional problems; 3) limitations in usual role activities because of physical health problems; 4) bodily pain; 5) general mental health (psychological distress and well-being); 6) limitations in usual role activities because of emotional problems; 7) vitality (energy and fatigue); and 8) general health perceptions. The primary advantage of the WOMAC questionnaire is its disease-specific nature supported by studies suggesting it is more sensitive to change and has greater efficiency for the study of osteoarthritic function than other questionnaires measuring overall health-related quality of life (21;34;38;74;75). However, the use of a generic, non disease specific measure of a patient's health such as the SF-36 is also important as it measures other important aspects of health not considered in the WOMAC (34). Its main use is to allow the comparison of health related quality of life across different diseases (i.e. osteoarthritis and heart disease) thus allowing the impact of disease to be compared and contrasted across diseases.

Those individuals who did not present with any symptoms and entered the study as a "healthy individual" but who showed radiographic evidence of disease on X-ray did not complete the questionnaires. Thus, only those individuals who claimed to have been diagnosed with knee OA upon entering the study completed both the WOMAC and SF-36 questionnaires (N=41, females = 26, males = 15). For the females, the group's mean (SD) age, height, weight and BMI were 61.1 (10.9) years, 160.5 (7.4) cm, 73.3 (13.0) kg and 28.6 (5.6) kg/m^2, respectively. Corresponding data for the males were 63.1 (9.7) years, 178.9 (7.7) cm, 88.8 (14.5) kg and 27.8 (3.7) kg/m^2, respectively. Kellgren-Lawrence grading of the females' radiographs revealed that 2 were grade 0, 8 were grade 1, 6 were grade 2, 5 were grade 3 and 5 were grade 4. Likewise for the males, 1 was grade 0, 2 were grade 1, 3 were grade 2, 3 were grade 3 and 6 were grade 4. Using scores from the two questionnaires, two different relationships were explored; 1) that between disease severity measured by the K-L score and symptoms of pain, stiffness and disability and 2) that between mJSW values and symptoms of pain, stiffness and disability.

Shown in Figures 18, 19 and 20 are the relationships between radiographic evidence of OA severity and symptoms of pain, stiffness and limitations in daily physical functioning. In general, these results demonstrate that increasing K-L grade (increasing disease severity) is

associated with increasingly severe symptoms of pain and stiffness and increased difficulty performing daily physical functions. Similarities between scores of those with K-L grades of 1 and 2 may indicate that those with a grade of 1, defined as "doubtful narrowing of joint space and possible osteophytic lipping" may indeed have very early knee OA represented by an onset of clinical symptoms before a presence of osteophytes or joint space narrowing can be seen radiographically. In addition, the subjectivity introduced by the K-L grading system and the lack of a clear definitive distinction between the meanings of K-L grades 1 and 2 may also explain this disparity in results.

To further explore the relationship between symptoms and radiographic evidence of knee OA, correlation analyses were conducted to determine if there was a significant association between measures of pain, stiffness and functional capacity and mJSW in the medial tibiofemoral compartment of the knee. As shown in Table 8, Pearson correlation coefficients and associated 95% confidence intervals revealed that pain, functional ability and total WOMAC scores were significantly related to mJSW in osteoarthritic females. In males, however, only functional ability and total WOMAC scores were significantly related. However, due to the relatively small numbers of individuals in each of the groups, wide confidence intervals were revealed. For instance, associated r^2 values ranged from 2% to 55% for the correlation between pain and mJSW in

women. The corresponding interpretation of such values means that between 2 and 55% of the variation in mJSW can explain the variation in pain felt by osteoarthritic females. Similar r^2 values are associated with functional ability and total WOMAC score in females. In males, r^2 values ranged from 5 to 80% for the total WOMAC score and these values were similar for functional ability. While these results do, indeed, suggest that those with narrower spaces between the distal femur and the proximal tibia in the medial compartment of the knee have more severe pain and more trouble performing daily functions (i.e., walking down stairs, performing household chores, getting out of bed, etc.) than those with larger spaces, the wide confidence intervals and associated r^2 values make it difficult to determine exactly what proportion of these symptoms can be accounted for by mJSW measurements. To improve these results and narrow the confidence intervals, larger sample populations would be required.

The relationship between mJSW and the 8 variables contained within the SF-36 were also investigated in this population by performing correlation analyses. Of these variables, only the physical function score related significantly to mJSW in both sexes. In females, the Pearson correlation coefficient was 0.59 (95% CI: 0.27, 0.80) and in males it was 0.63 (95% CI 0.17, 0.86). Once again, these results suggest that those with a smaller mJSW had more limitations in physical function due to

problems in health than those with larger mJSW. However, the presence of large confidence intervals once again makes it difficult to determine the proportion of functional limitations that can be accounted for by variations in mJSW. Regression analyses investigating the relationship between mJSW and the other measures of overall general health and well-being were not significantly related to mJSW in either males or females ($p>0.05$). These results are what would normally be expected since a generic health-related quality of life questionnaire is not designed to measure change in all domains in specific diseases. Specific domains would be expected to demonstrate change as in the physical function domain. The measurement of domains that are considered directly relevant to OA justified the development of the WOMAC questionnaire.

Chapter 4: Bone Mineral Density Results

4.1 Quantification of Bone Mineral Density in the Lumbar Spine, Proximal Femur, Distal Femur and Proximal Tibia Regions in Healthy Volunteers

Of the 120 healthy individuals who underwent a knee X-ray, 94 also underwent DXA scans of their lumbar spine, hip, distal femur and proximal tibia. Those 26 individuals who did not participate in the DXA portion of the study were those who had participated prior to including the BMD measurements in the protocol. Fifty-seven of the 94 volunteers were females while the remaining 37 were males. Descriptive results of the lumbar spine and total hip bone density analyses are displayed in Table 9. Graphically, the relationship between age and BMD in the spine is displayed in Figure 21 while the relationship between age and total hip BMD is shown in Figure 22. It appears that BMD does not change significantly with age in healthy volunteers. It also appears that healthy men have denser bones than healthy women of similar ages. To test these two observations, backwards linear regression analyses were performed. Age and BMI were inserted into the analyses as independent variables with the dependent variables being BMD in the L-spine and total hip regions. For neither males nor females was age significantly related to BMD ($p>0.05$). However, these same analyses supported the notion that BMI is significantly related to BMD in the L-spine and total hip in healthy

females ($p<0.05$). The β-coefficients for the L-spine and hip regions were 0.471 and 0.514, respectively, suggesting that those with a lower BMI have less dense bones while those with a higher BMI have more dense bones. This relationship was not observed in healthy males. When gender was included with age and BMI as independent variables, it was revealed that, indeed, BMD in the L-spine and total hip was significantly higher in males than in females ($p<0.05$). It is plausible that BMD may have been falsely elevated in older subjects due to degenerative changes.

Bone mineral density was also analysed in the distal femur and proximal tibia as per the protocol previously described. Those regions which were especially of interest were those in the subchondral region, directly adjacent to the articular cartilage, F4 and T1 in Figures 8 and 9 respectively, and those which incorporated all 4 regions in each of the distal femur and proximal tibia which will be referred to as "total distal femur" and "total proximal tibia" for the sake of simplicity. The mean, standard deviation, range, minimum and maximum BMD values for healthy males and females in these regions of interest are displayed in Table 10. It should be noted that BMD values in the distal femur and proximal tibia were unable to be analyzed in 2 fewer healthy males and 2 healthy females than in the regions of the L-spine and total hip. Unfortunately, these scans were not saved and archived after they were acquired and thus were deleted from the scanning computer. In addition,

tibial scans from one healthy male and one healthy female were not able to be analyzed due to inadequate positioning during scan acquisition.

Upon dividing the healthy population into age groups, BMD in the total distal femur and total proximal tibia were analyzed and results are shown graphically in Figures 23 and 24. Linear regression analyses were implemented to determine whether or not age group, as a categorical variable, or age, as a continuous variable, was significantly related to BMD in either the total distal femur or the total proximal tibia. Figures 23 and 24 show that age group was not significantly related to BMD in either of these areas of interest and there was no significant difference in BMD between any age group as determined by ANOVA analyses ($p=0.459$). In considering age continuously, regression analyses showed that in females, BMI was significantly related to BMD in both the distal femur and proximal tibia suggesting that those who are heavier set (higher BMI) have more dense bones in these areas. The slope of the regression line (β-coefficient) for the distal femur was 0.378 and 0.593 for the proximal tibia ($p<0.05$). Age was also found to be significantly related to BMD in the proximal tibia in healthy females with a β-coefficient of -0.301 ($p<0.05$). In healthy males, these same analyses were conducted, yet neither age nor BMI was found to correlate significantly with BMD in the thigh or leg.

To determine whether there was a significant difference in distal femoral and proximal tibial BMD between healthy males and females, gender was independently inserted into a backwards linear regression model. Gender was significantly related to distal femoral BMD with a β-coefficient of 0.388 suggesting that males have denser bones in the axial skeleton than females. This was also the case when total proximal tibial BMD was analyzed. Once again, gender was a significant predictor of proximal tibial BMD as shown by a β-coefficient of 0.299. These results are consistent with those observed for the regions of the hip and spine.

4.2 Quantification of Bone Mineral Density in the Lumbar Spine, Proximal Femur, Distal Femur and Proximal Tibia Regions in Volunteers with Knee OA

The same 33 osteoarthritic females who underwent a knee X-ray also underwent BMD scans of their spine, hip, femur and tibia. Of the 22 osteoarthritic males who had a knee X-ray, 20 of them also had BMD scans. Those 2 who did not undergo DXA scans were those who had only participated in the X-ray portion of the study. Thus, in 53 volunteers with knee OA, BMD in the L-hip and spine were quantified and descriptive statistics are displayed in Table 11. The relationship between age and BMD in these regions was also investigated and is shown graphically in Figures 25 and 26. Although there did not appear to be a significant

relationship between these variables in healthy individuals, backwards linear regression analyses were used to determine if age and BMI were significantly related to L-spine and hip BMD in males and females with knee OA. As shown in Table 12, both BMI and age were significantly related to BMD in the spine and hip in females, although the same was generally not true for males. Regression analyses performed to test whether the presence of knee OA, BMI, age and gender were significantly related to spine and hip BMD revealed that only the presence of knee OA was not found to be significantly related to bone density. In other words, there were no significant differences in BMD between males and females without knee OA compared to those with OA. Descriptive data of these two groups support this finding (Tables 9 and 11). Men with OA were found to have significantly denser bones than women with OA (β-coefficient 0.240), as did those who were younger and had a higher BMI as shown by β-coefficients of 0.324 and -0.207, respectively ($p<0.05$), in the L-spine. However, the possibility of these results being due to the presence of osteophytes and degenerative disc disease cannot be ruled out. Nevertheless, these results were consistent with those yielded from analyses in the hip in which gender, BMI and age were once again significantly related to BMD as shown by the following β-coefficients; 0.355, 0.373 and -0.365. Due to the small numbers of individuals in the OA groups, specifically those age groups <50 yrs, bone density data as it relates to age group was not analyzed.

Bone mineral density in the distal femur and proximal tibia were also analyzed in osteoarthritic males and females. One less distal femoral and two less tibial scans were analyzed in osteoarthritic females because, once again, the scans were not properly archived. Of those scans that were acquired and analyzed, descriptive statistics are displayed in Table 12. Regression analyses were performed to identify whether gender or presence of knee OA was significantly related to BMD in any of the regions of interest listed in Table 13. Results revealed that there were no significant differences in BMD in the distal femur or the proximal tibia between those with knee OA and those without in either males or females ($p>0.05$). There were significant differences, however, between BMD in the distal femur and proximal tibia in osteoarthritic males compared to females. Consistent with previously reported results, males, once again, had significantly higher BMD than females when comparing the same regions of interest ($p<0.05$) (60). β-coefficients for the 4 regions of interest ranged from 0.291 to 0.506.

To investigate whether age and BMI were significantly related to BMD in the 4 regions of the distal femur and the proximal tibia, backwards linear regression analyses were conducted. Results of these analyses, displayed in Table 14, were consistent with those in healthy individuals and also those in osteoarthritic individuals in the regions of the L-spine and hip. Both BMI and age were significantly related to proximal tibial and

distal femoral BMD in females with OA with the exception of age being significantly related to BMD in the subchondral region of the distal femur. Generally speaking, however, the positive regression slope for BMI suggests that denser bones are associated with those who are heavier while the negative regression slope for age indicates BMD in the distal femur and proximal tibia is less in those who are older than those who are younger. Unfortunately, however, these same relationships were not observed in males. Neither age nor BMI were found to significantly relate to BMD in the distal femur or the proximal tibia, possibly because of the small number of males (n=20) who underwent these scans and the inadequately powered sample size. Once again, age group specific analyses were not conducted because of the small number of osteoarthritic individuals in many of the age groups.

Since age and increasing disease severity often parallel one another, correlation analyses were performed to test whether the severity of knee OA, as graded on the K-L scale, was related to BMD in the L-spine, total hip, distal femur and proximal tibia. Results of these analyses revealed that BMD in the subchondral regions of the distal femur (F4) and proximal tibia (T1) were, indeed, significantly related to disease severity with correlation coefficients of 0.352 and 0.284, respectively ($p<0.05$). Graphical representations of the distribution of BMD to K-L grade are shown in Figures 27 and 28. Bone density in the total distal femur, total

proximal tibia and the L-spine and hip regions of the body were not found to be significantly correlated with disease severity as graded by Kellgren-Lawrence.

Chapter 5: Magnetic Resonance Imaging Results

5.1 Inter-rater Reliability of Cartilage Volume and Thickness Measurements Using the Cartilage Segmentation Algorithm

To test the inter-rater reliability of the cartilage measurements acquired using the manual segmentation algorithm, images from 28 of the 138 knee images were analyzed a second time by an independent technician in Salzburg, Austria. Acquired from 18 female and 10 male participants, the mean (SD) age and BMI of the volunteers in this sample were 66.5 (8.9) yrs and 29.1 (5.0) kg/m^2, respectively. This sample was comprised primarily of knee OA patients (82%) as defined by their K-L gradings; 6 were K-L 2, 7 were K-L 3 and 10 were K-L 4. The remaining 5 patients (18%) were K-L grades 0 and 1 representing an absence of radiographic OA. The selection of participants for the reliability test was random, although the proportion of healthy and knee OA volunteers in the sample was not. Due to the fact that images from OA patients are more challenging to analyze because of the presence of osteophytes and denuded areas of cartilage, the large percentage of OA individuals in the sample was chosen to attempt to give a conservative estimate of reliability for the study.

After being analyzed in Hamilton, raw images were sent to the Paracelsus Medizinische Privatuniversität in Salzburg, Austria. Upon receiving the DVD, one technician analyzed the medial tibial

compartments of all 28 images using the identical version of the proprietary software program. This technician was blinded as to the age, BMI, gender, K-L grade and, of course, measurements of cartilage volume and thickness yielded in Hamilton. After completing the analyses, segmented images as well as an Excel file of the resultant data were sent back to Hamilton for comparisons to be made. Cartilage volume and thickness measurements for these 28 individuals were entered into the SPSS patient database where intra-class correlation coefficients and coefficients of variation were calculated.

Measurements of medial tibial cartilage volume and thickness were compared between the two analysts. Intra-class correlation coefficients (ICC) and coefficients of variation (CV) were similar between cartilage volume and thickness measurements. Results of these analyses are shown in Table 15 where all coefficients were found to be significant at the 5% level ($p<0.05$). Analyses were repeated for only the 5 healthy individuals and results revealed a CV of 15.3% for cartilage volume and a CV of 11.8% for thickness, results which are slightly better than for the entire group together.

5.2 Quantification of Medial Tibial Articular Cartilage Volume and Thickness in Healthy Volunteers

Of the 94 healthy individuals who underwent a knee X-ray and DXA scans, 86 of these, 50 females and 36 males, also agreed to have an MRI scan of their same knee. The mean (SD) age was 39 (13) years and the mean BMI was 25 (5.2) kg/m^2 for the females. For the males, the mean age (SD) and BMI were 37 (14) years and 25 (3.2) kg/m^2, respectively. MR images were analyzed using a software algorithm to quantify articular cartilage volume and thickness in the medial tibia. An image of an analyzed medial tibia is displayed in Figure 29. Data representing the average medial tibial cartilage volume and thickness results, as well as the range, minimum and maximum values, are presented in Table 16. Linear regression analyses were performed to confirm the apparent difference in volume and thickness between males and females. Indeed, males had greater volume and thicker cartilage than females ($p<0.05$). To investigate the relationship between age and cartilage volume and thickness, graphs of these variables versus age were drawn and are represented in Figures 30 and 31. Linear regression analyses were conducted to better understand these relationships. In females, it was seen that both age and BMI were significantly related to medial tibial cartilage volume with β-coefficients of -0.407 and 0.296, respectively ($p<0.05$). In males, however, neither variable was significantly related to articular cartilage volume. The case was similar for medial tibial articular cartilage thickness

where only age was found to significantly relate in healthy females (β-coefficient -0.374, $p<0.05$) but BMI was not. Once again, in males, no significant relationships between age and BMI and cartilage thickness were identified. The presence of a small sample size in the male subject group may be a contributing factor to the absence of statistically significant relationships.

Medial tibial articular cartilage volume and thickness were also determined for each decade of life in healthy males and females. Figures 32 and 33 represent the distribution of mean volume and thickness in each age group and appear to show that, in healthy females, articular cartilage volume and thickness decrease consistently with increasing decades. In males, however, this does not seem to be the case. In fact, cartilage volume and thickness in the medial tibia even appear to increase between the 5^{th} and 7^{th} decades of life. However, these unexpected trends can likely be explained, once again, by the small numbers of individuals in each of these groups. For instance, in the 40-49, 50-59 and 60-69 year old groups there were only 3, 6 and 3 individuals, respectively. Thus, it is possible that increasing the sample sizes might lead to expected trends in cartilage volume and thickness such as those seen in females. ANOVA analyses were conducted to determine if there were significant differences in cartilage volume and thickness between age groups in both males and females. Results of these analyses showed

that, in fact, there were no statistically significant differences between age groups ($p<0.05$) suggesting that older individuals do not have significantly less cartilage or significantly thinner cartilage than those in earlier decades. However, when considering both age group and gender in the ANOVA analyses, the interaction between these two variables showed that, indeed, males had more cartilage volume and thicker cartilage than women in the same decade of life ($p<0.05$). Once again, these results support those observed in mJSW and BMD analyses of differences between males and females.

5.3 Quantification of Medial Tibial Articular Cartilage Volume and Thickness in Osteoarthritic Volunteers

MR images of the knees of 33 females and 22 males with OA were acquired. Images from 1 female participant were not saved in adequate form and were not able to be analyzed. In addition, 2 osteoarthritic males who underwent knee X-rays did not participate in the MRI portion of the study as one had undergone angioplasty and did not feel comfortable entering the imaging area and the other was not able to be contacted to return for the MRI portion of the study. Images from the remaining 52 osteoarthritic individuals were analyzed using the cartilage segmentation program to quantify articular cartilage volume and thickness in the medial tibial compartment, two examples of which are shown in Figures 34 and

35. In addition to these variables, the percentage of subchondral bone covered with articular cartilage and the percentage of subchondral bone area denuded by cartilage were also quantified for this population. Descriptive statistics of these variables in this population are displayed in Table 17. Regression analyses were conducted to determine if there were significant differences between the volume of cartilage normalized to bone surface area and articular cartilage thickness between males and females. In fact, no significant differences were detected ($p>0.05$) suggesting that males affected by knee OA do not have significantly less cartilage or thinner cartilage than females with knee OA.

In investigating the relationship between age and cartilage volume and thickness, graphs of the relationships between these variables were prepared and are shown in Figures 36 and 37. Backwards linear regression analyses were also performed to determine the magnitude and the strength of the relationship between age and BMI and medial tibial articular cartilage volume and thickness. In osteoarthritic females, both age and BMI were found to be significantly related to cartilage volume (normalized to bone surface area) with β-coefficients of -0.413 and -0.398, respectively ($p<0.05$). These results suggest that aging and increasing weight in proportion to height is associated with smaller cartilage volume. In males, aging was also significantly related to decreasing cartilage volume as suggested by a significant β-coefficient of -0.476 ($p<0.05$).

These results were almost exactly duplicated when analyses were performed to investigate the relationship between age and BMI and medial tibial cartilage thickness. In females, β-coefficients were -0.436 and -0.401 for age and BMI, respectively, and -0.480 for age in males ($p<0.05$).

Perhaps more meaningful than the relationship between age and cartilage volume and thickness is the relationship between disease severity, as evaluated by the K-L scale, and cartilage volume and thickness. Because age and disease severity are often but not necessarily correlated with one another (i.e. a person in his/her fourth or fifth decade of life may have more severe disease than someone in his/her sixth or seventh decade), an investigation was performed to evaluate the relationship between K-L grade and medial tibial cartilage volume and thickness in males and females. As shown in Figures 38 and 39, there is an apparent consistent decrease in both volume and thickness with increasing K-L grade in both males and females. Linear regression analyses adjusting for age and BMI revealed that K-L grade was significantly related to medial tibial cartilage volume normalized to bone area with β coefficients of -0.580 and -0.547 for females and males, respectively. Likewise, K-L grade was also significantly related to cartilage thickness in the medial tibia with β coefficients of -0.601 and -0.368 for females and males, respectively.

5.4 Relationships between Medial Tibial Articular Cartilage Volume and Thickness and Pain and Function in Osteoarthritic Volunteers

The relationship between subcategories of the WOMAC questionnaire and the SF-36 and cartilage volume and thickness were investigated as was the relationship with mJSW in Chapter 3. As previously discussed, the WOMAC questionnaire was subdivided into 3 different areas, pain, stiffness and physical function, as well as the total score. The same 41 individuals who were included in the analyses with mJSW were included here. The group's mean (SD) age, height, weight and BMI for the group were 64.8 (10.3) years, 167.0 (11.4) cm, 78.9 (16.1) kg and 28.3 (5.0) kg/m^2, respectively. Mean (SD) cartilage volume and thickness values were 1.50 (0.65) mL and 1.19 (0.45) mm, respectively. Correlation analyses between medial tibial cartilage volume and thickness and subcategories of the WOMAC and SF-36 questionnaires were performed. Results, as presented in Table 18, show that all aspects of the WOMAC questionnaire were significantly correlated to medial tibial cartilage volume and thickness. In fact, cartilage thickness measurements correlated more strongly than volume measurements with all pain and function parameters. Thus, as cartilage volume decreases and becomes thinner, significantly more pain and stiffness and decreased physical functional capacity are experienced by the individual.

Relationships between medial tibial cartilage volume and thickness and all measures of the SF-36 were also investigated. Just as seen when investigating the relationship with mJSW, both cartilage volume and thickness were found to correlate significantly ($p<0.05$) with limitations in physical functioning as shown by Pearson's correlation coefficients in Table 18. Despite the non-specific nature of the SF-36 questionnaire with respect to the disease or condition affecting the patient, significant relationships between limitations in physical function correlated with variables related specifically to knee osteoarthritis.

5.5 Abnormalities Identified in the Knees of Healthy Volunteers Using MRI

After acquiring numerous MR images of healthy knees for the purpose of quantifying "normal" ranges of articular cartilage volume and thickness in males and females of various ages, it became evident that it was possible to visualize bone and soft-tissue abnormalities present in the in these same images. Thus, in addition to performing quantitative analyses, the opportunity arose to semi-quantitatively evaluate various other abnormalities in this population giving rise to estimates of the prevalence of bony, ligamentous, meniscal and cartilaginous defects in healthy individuals. Using MR images acquired from a small subset of the

entire "healthy" study population, an additional sub-study was conducted (76).

The first 44 healthy individuals to undergo a knee MRI were those who comprised the study group. Of these volunteers, 33 were female and 11 were male with a group mean (SD) age of 41.1 (14.2) years (range 20 to 68 years), and a group mean (SD) BMI of 25.4 (4.4) kg/m^2. Kellgren-Lawrence scoring of these volunteers' plain X-rays revealed that 29 patients (66%) were grade 0, 12 (27%) were grade 1 and 3 (7%) were assigned grade 2.

Two radiologists (Dr. Margaret Pui and Dr. John O'Neill) independently examined the 44 MR images and evaluated the presence and severity of various soft tissue and bony features in and around the knee joint. The grading of these images was recorded on a semi-quantitative scoring sheet as shown in Table 19. In the case that the two radiologists did not agree on a score assigned to a particular feature, both radiologists reviewed the study and a consensus grade was assigned.

Although no bone marrow edema or ligamentous lesions were found in the knees of any participants, subchondral cysts were identified in 6 different individuals (13.6%), 5 of whom were K-L grade 0 while the other was grade 2. Of these six individuals, 5 were over the age of 53

years while one was 35 years old. Grade 1 cartilage lesions were identified in 5 different individuals (11.4%) in various regions of the knee, 4 of whom were between the ages of 60 and 68 years while the other was 41 years old. Only one of the three individuals with K-L scores of 2 exhibited a cartilage lesion while one individual with K-L grade 1 demonstrated cartilage lesions at every location of the knee. Articular cartilage in the regions of the femoral trochlea, medial femur and the patella was most commonly found to have low signal intensity. The prevalence of cartilage lesions in various regions of the knee are shown in Table 20.

Osteophytes and meniscal abnormalities were the most prevalent abnormalities identified in this asymptomatic population. Twelve individuals (27.3%) ranging in age from 22 to 58 years were identified as having grade 1 osteophytes on pMRI, the regions most commonly affected being the tibial spines, femoral trochlea and patella as shown in Table 20. All individuals with a K-L score of 2 (evidence of osteophytes) on plain radiographs also showed evidence of osteophytes on pMRI. Five individuals with a K-L grade of 0 and 4 with a K-L grade of 1 comprised the additional 9 individuals who were identified as having osteophytes present on pMRI. Participants identified as having osteophytes did not appear to be more likely to have meniscal defects or cartilage lesions than those that did not have osteophytes.

Meniscal abnormalities were also prevalent in this asymptomatic population as shown in Table 21. Of 44 participants, only one individual showed no evidence of a meniscal abnormality in any region of the knee. Six individuals had an abnormality in 1 region, 10 showed evidence of an abnormality in 2 regions and the remaining 27 individuals (61.4%) showed evidence of meniscal abnormalities in ≥3 of the four regions of the knee. The majority of these abnormalities were present in individuals whose knees were considered to be "healthy" (K-L grade 0). Of the abnormalities in the medial menisci, 87% were considered degenerative changes (grade 1) while remaining 13% were meniscal tears (grade 2). Likewise, 93% of the abnormalities observed in the lateral meniscus were grade 1 degenerative changes while 7% were grade 2 meniscal tears.

Both the anterior and posterior horns of the medial meniscus were most commonly found to have a minimum of a grade one abnormality indicative of degeneration with prevalences of 70% (31/44) and 89% (39/44), respectively. In assessing the anterior and posterior horns of the medial and lateral menisci individually, 39 individuals (89%) were identified as having a meniscal abnormality in the anterior horn of either one or both of the menisci while 43 individuals (98%) had a posterior horn abnormality. Despite the relatively small sample size resulting in few individuals in the 60-69 year old age group (N=5), the relative percentages of grade 1 meniscal degeneration in each of the four regions examined

appeared to increase with increasing decade of life. Examples of this are shown in Figures 40 and 41.

Chapter 6: Results of Relationships between Outcome Variables

Relationships between mJSW, Bone Mineral Density and Articular Cartilage Volume and Thickness in Healthy Volunteers

6.1 Minimum Joint Space Width and Bone Mineral Density:

To date, relationships between mJSW in the knee, specifically the medial compartment, and BMD in the hip, spine, distal femur and proximal tibia have not been explored extensively in either healthy or osteoarthritic individuals. In order to understand relationships among variables in those with OA, it is important to establish an understanding in those who are "normal" for reference and comparison purposes. Initially, these relationships were explored and analyses were performed in healthy females and males, separately, and then together.

To determine if mJSW in the medial tibia was related to total L-spine and total hip BMD (same side), backwards linear regression analyses were performed. Age, BMI and mJSW were all considered independent variables with BMD the dependent variable. In females, at both the hip and spine, mJSW was significantly related to BMD ($p<0.05$) with β-coefficients ranging from 0.244 to 0.263. Thus, in females, these results suggest that those with separations between the femur and tibia

have more dense bones than those who are smaller in stature and have narrowed joint spaces. In males, a significant relationship between mJSW and total hip BMD was identified (β-coefficient=0.295, $p<0.05$), although mJSW was not found to be significantly related to lumbar spine BMD. Upon repeating these analyses for females and males together as a single population, results similar to those in females were found. Once again, mJSW data were significantly related to BMD in both the L-spine and total hip, although the slope of the line was steeper for the total hip as reflected by a higher β-coefficient (0.250 at the L-spine and 0.408 at the hip). As anticipated, when considering males and females together, values of the β-coefficients decreased relative to those in females because of the non-significant values in males. However, it is important to note the direction of these relationships suggesting the presence of positive, significant correlations between BMI and mJSW while spine and hip BMD remained constant with age.

Relationships between mJSW and BMD at the distal femur and proximal tibia were also investigated in males and females. Regions of the distal femur and proximal tibia considered in the analyses were "total" subchondral bone density, reflecting a mean density of the 4 regions of interest as shown in Figures 8 and 9, and the subchondral regions labeled as F4 and T1. Results of backward linear regression analyses revealed that mJSW was not significantly related to BMD in the total distal femur or

the subchondral femoral region in males or females. However, in contrast to the femur, mJSW was significantly related to BMD in the two regions of the tibia under investigation. In relation to the mean BMD in the entire proximal tibia, the β-coefficient for mJSW was 0.265 while in the subchondral region of the tibia (T1) it was 0.255 ($p<0.05$). Once again, no significant relationships between mJSW and BMD in the proximal tibia were identified in males.

Backwards linear regression analyses were repeated when males and females were considered one group. In contrast to results in both males and females when analyzed independently, medial mJSW was found to relate significantly to BMD in the total distal femur when the groups were considered together (β-coefficient = 0.421). In the total proximal tibia and in the subchondral tibial region, mJSW was found to correlate significantly with BMD as revealed by β-coefficients of 0.408 in the proximal tibia, and 0.311 in the subchondral region ($p<0.05$). Thus, in normal subjects, a greater mJSW is associated with denser bones in the regions surrounding the knee joint in the same manner as mJSW is related to BMD in the lumbar spine and proximal femur.

6.2 Minimum Joint Space Width and Articular Cartilage Volume and Thickness:

Minimum joint space width has long been thought to be a surrogate measure of articular cartilage in the knee joint. Intuitively, those who have thick cartilage would have greater joint space width than those who have thin cartilage. In fact, in more severe cases of knee OA, cartilage degeneration eventually leads to denuded areas reflecting direct bone on bone contact and a resultant mJSW measurement of 0 mm. However, mJSW measurements reflect the sum of femoral cartilage thickness, tibial cartilage thickness as well as other possible soft tissue contributions such as from menisci. What remains unknown is the proportion of mJSW accounted for by the thickness of cartilage in each region. Thus, analyses were performed to determine the association between mJSW in the medial compartment of the knee and cartilage thickness in the medial tibia.

To determine this, linear regression analyses accounting for differences in age and BMI were performed to evaluate the relationships between both cartilage volume and cartilage thickness and mJSW in healthy males and females. Since neither age nor BMI were significantly related to cartilage volume or thickness, they were not considered as covariates in the analyses and regression coefficients can be expressed as Pearson correlation coefficients. Indeed, cartilage volume and

thickness were significantly related to mJSW with β-coefficients being 0.513 and 0.545 for females and 0.581 and 0.624 for males, respectively ($p<0.05$). Considered together as a healthy population, the β-coefficients reflecting the relationship between mJSW and mean cartilage thickness over total bone and mJSW and cartilage volume were greater at 0.686 and 0.666, respectively ($p<0.05$). Calculations of confidence intervals showed that there were no significant differences between any of the β-coefficients representing the relationships between these variables because of their overlap. Table 22 shows the relevant correlation coefficients, their respective 95% confidence intervals and r^2 values.

Three observations appear evident from these results. As expected, cartilage thickness is more strongly related to mJSW than is cartilage volume for both males and females and together as a whole. The second observation is that the relationship is stronger in males than in females. However, when considered a "healthy" population (males and females together), the strength of this relationship appears greatest as reflected by the magnitude of the r values. For instance, the r^2 values suggest that 39% of the variation in mJSW in the medial tibia can be explained by the variation in medial tibial cartilage thickness in males. In females, only 30% of the variation in mJSW is explained by the variation in cartilage thickness while in general, 47% is explained in both males and females. A graph depicting the latter relationship is displayed in Figure

42. The third key observation here reveals that, despite these seemingly apparent differences, 95% confidence intervals suggest no significant differences exist between the correlations coefficients in males and females without knee OA. The issue that predominates is the question of what variable/variables account(s) for the remaining 61-70% of the variation in mJSW that is not accounted for by variation in tibial cartilage thickness. While it is possible that femoral cartilage thickness and medial meniscal subluxation may be valid contenders, these data and results cannot confirm this.

6.3 Bone Mineral Density and Articular Cartilage Volume and Thickness:

Two different relationships between BMD and cartilage volume and thickness were analyzed, the first of which focused on the relationship between BMD in the lumbar spine and cartilage volume and thickness in the medial tibia. The second was the relationship between BMD in the distal femur and proximal tibia and cartilage volume and thickness in the medial tibia. The results will help to determine if BMD around the affected joint is influenced by knee OA or whether, in fact, BMD throughout the core skeleton is affected by OA in the knee, suggesting that bone is affected systemically rather than locally. Backwards linear regression analyses accounting for age and BMI as covariates were performed in

males and females to demonstrate if such relationships existed in healthy individuals. Results of these analyses showed that, in healthy women, lumbar spine BMD was significantly related to cartilage volume and thickness in the medial tibia with β-coefficients between 0.321 and 0.378, respectively ($p<0.05$). In fact, the larger coefficient demonstrates that cartilage thickness had a slightly stronger relationship with L-spine BMD than did volume. Interestingly, L-spine BMD was not significantly related to either cartilage volume or thickness in healthy males. In combining males and females, once again, L-spine BMD was significantly related to both variables with β-coefficients between 0.276 and 0.308 ($p<0.05$), respectively, albeit with noticeably smaller coefficients than women alone. Interpreting these results suggests that those with lower bone density in the L-spine also have less cartilage and thinner cartilage in the medial tibia compared to those who have more dense bones. Females, who typically have lower bone densities than males also have a higher incidence and prevalence of knee OA in the population. One might propose that these results may suggest another possible explanation as to why females are affected by OA to a much greater extent than males.

Regression analyses were also conducted to determine the presence of significant relationships between medial tibial cartilage volume and thickness and its surrounding bone in the distal femur and proximal tibia. Intuitively, one might think that "healthy" bones surrounding

a joint may be correlated with cartilage parameters because they are physically in contact and have the ability to influence the cellular activity occurring in one another. As with previous analyses, the mean BMD in the regions of the femur and tibia as well as the subchondral regions (F4 and T1) were considered as independent variables with age and BMI included as covariates. Significant relationships were identified between total femoral BMD and total tibial BMD and cartilage volume and thickness. While β-coefficients representing the strength and direction of the regression lines ranged from 0.292 to 0.323 for the distal femur, those for the proximal tibia were higher at 0.369 to 0.379 ($p<0.05$). These results support the notion that cartilage in the medial tibia is more closely related to the bone adjacent to it in the proximal tibia than the bone which lies superior to it in the femur. Consistent with results from the lumbar spine, those with higher BMD appear to have more cartilage overall, and thicker cartilage. Relationships between only the subchondral regions of BMD and cartilage parameters were not statistically significant. The results of the investigation of the relationship between distal femoral and proximal tibial BMD in males revealed no significant correlations, consistent with the L-spine results. Bone mineral density in the subchondral region of the distal femur and proximal tibia was not related to medial tibial cartilage volume or thickness in either males or females. Although this may appear to be inconsistent with intuition, the lack of

significant relationship may reflect a lack of precision in subchondral BMD measurement rather than reflecting a true value of the relationship.

Relationships between mJSW, Bone Mineral Density and Articular Cartilage Volume and Thickness in Volunteers with Knee OA

6.4 Minimum Joint Space Width and Bone Mineral Density:

The relationships between medial mJSW and BMD in the lumbar spine, proximal femur, distal femur and proximal tibia were investigated in osteoarthritic individuals just as they were in those without knee OA. Once again, age and BMI were considered independent variables in the analyses, along with mJSW, because of their abilities to impact both mJSW and BMD independently. Backwards linear regression analyses in females and males revealed that mJSW was not significantly related to L-spine or hip BMD in those with knee OA as was observed in healthy individuals. These results were reproduced consistently when the relationships were analyzed for one group of osteoarthritic individuals (i.e., males and females together).

In exploring the relationship between mJSW and BMD in the distal femur and proximal tibia of osteoarthritic participants, backwards linear regression analyses were repeated. Results of analyses conducted to

investigate the relationships between the independent variables and subchondral BMD in the distal femur and proximal tibia revealed that, in osteoarthritic females, mJSW was significantly related to BMD in the F4 region (Figure 8), but not the total distal femur, as shown by a β-coefficient of -0.644 ($p<0.05$). This was also seen in males with knee OA where the β-coefficient representing the slope of the regression line between mJSW and F4 bone density was -0.582. However, BMD in the total proximal tibia and in the T1 region was not significantly related to mJSW in either males or females ($p>0.05$). It is important to note here, that the relationship between mJSW and BMD in the distal femur of osteoarthritic individuals is the reverse of that observed between mJSW and BMD in healthy individuals. In addition, mJSW was found to be significantly related to total proximal tibial and subchondral tibial BMD in healthy individuals but not in those with knee OA.

In performing these statistical analyses in males and females together, the slope of the regression line representing the relationship between subchondral femoral BMD and mJSW was -0.426, suggesting that smaller mJSW measurements are associated with higher bone density in and around the subchondral region of the femur in those with knee OA. The direction of this relationship is opposite to that seen in healthy individuals and consistent with previously published data.

6.5 Minimum Joint Space Width and Articular Cartilage Volume and Thickness:

As previously alluded to, the notion that mJSW is a surrogate measure of cartilage thickness suggests that there should be a strong, positive relationship between these variables in osteoarthritic individuals. As medial tibial cartilage becomes thinner over time, so too should the space between the distal femur and proximal tibia in the medial compartment. While accounting for differences in age and BMI, regression analyses performed to determine the strength of this relationship supported this point, although, once again, the relationship between variables was stronger in osteoarthritic males than females. In osteoarthritic females, the values reflecting the slope of the regression lines for the relationship between cartilage thickness and volume and mJSW were 0.594 and 0.484, while, for males, the slopes were 0.880 and 0.756, respectively. Regression analyses for both males and females combined revealed β-coefficients of 0.720 and 0.498 for cartilage thickness and volume, respectively ($p<0.05$). Although the slopes of the regression lines reflecting the relationship between cartilage thickness and mJSW and cartilage volume and mJSW appear steeper in osteoarthritic males than females, the 95% confidence intervals suggest that these differences are not significant. However, the relatively small numbers of osteoarthritic males and females in these groups lead one to question

whether the sample size is sufficient to detect such differences between groups.

It is evident, by comparing the β-coefficients of osteoarthritic and healthy females (0.594 to 0.545), there was not a significant difference after accounting for differences in age and BMI. In addition, there was no significant difference between the slope of the regression line for healthy males (β-coefficient = 0.624) compared to healthy and osteoarthritic females. In other words, the slopes of the regression lines representing the relationship between medial tibial cartilage thickness and mJSW in healthy and osteoarthritic females and healthy males generally lie within ranges which overlap one another supporting the notion that they are not significantly different. However, the 95% confidence interval for the β-coefficient for the relationship between medial tibial cartilage thickness and mJSW in the medial compartment in osteoarthritic males (0.880) did not overlap those of the other groups. Thus, it appears there is a significantly stronger relationship between these variables in males with OA than in males without OA and females with or without knee OA.

Correlation analyses were also run to reveal, in another way, the results of the analyses of the relationships between mJSW and cartilage volume and thickness. Shown in Table 23, positive significant correlations were revealed in females and males. As in healthy individuals, mJSW

appeared to be more strongly related to cartilage thickness than volume and the strength of the relationships appeared greater in males than females. In males, 77% of the variation in medial tibial cartilage thickness explained the variation in medial mJSW, compared to only 53% in females. These results suggest that medial tibial cartilage may wear down to a greater extent in males than in females where a smaller proportion of the variation in mJSW could be explained by variation in medial cartilage thickness. When combined and looked at as an osteoarthritic population as a whole, 64% of the variation in mJSW is explained by the variation in cartilage thickness (Figure 43). This value is greater than the one shown in Figure 42 for healthy individuals where the r^2 value was 0.470 (47%). Unfortunately, however, the confidence intervals of the correlation coefficients shown in Table 23 are large and overlap one another suggesting that none of the apparent differences suggested above are statistically significant. In other words, cartilage volume measurements in the medial tibia are not more strongly related to mJSW measurements in males than in females or in osteoarthritic individuals compared to healthy ones. It is possible that, by increasing the numbers of individuals in each of the groups under investigation, these apparent differences may, in fact, be significant.

6.6 Bone Mineral Density and Articular Cartilage Volume and Thickness:

To investigate the notion that OA is a disease localized to a specific joint and not one that affects the entire musculoskeletal system, the relationship between L-spine BMD and medial tibial cartilage volume and thickness was analyzed using linear regression. In contrast to healthy females where these variables were found to be significantly related to one another, no significant relationships were identified in those with knee OA. Results in osteoarthritic males were consistent with those in healthy males where again, L-spine BMD had no significant relationship with volume and thickness parameters. When considered as an osteoarthritic population as a whole, males and females combined, the results did not change. Thus, these data support the notion that the skeletal effects of knee OA are not systemic but are localized to the bones surrounding the afflicted joint.

To test this hypothesis further, regression analyses were performed in an attempt to identify and/or quantify the relationships between subchondral bone density in the femur and tibia and medial tibial cartilage volume and thickness. Surprisingly, BMD in the entire distal femoral and proximal tibial regions was not significantly related to cartilage volume or thickness in the medial tibia in either males or females. Analyses of the subchondral regions yielded almost the same results, with one exception.

In males with knee OA, BMD in the subchondral region of the femur (F4) correlated significantly with both cartilage volume and thickness with β-coefficients of -0.713 and -0.814 ($p<0.05$). Perhaps the most important information revealed by these analyses is the direction of the relationship suggesting that higher BMD is associated with small cartilage volume and thin cartilage. This is opposite to that of the relationship derived from healthy females where high BMD in the total distal femur and proximal tibia was related to large cartilage volume and thickness. These data appear to support the notion that the relationship between BMD and cartilage volume and thickness appears opposite in healthy and osteoarthritic individuals.

Chapter 7: Discussion

The technique used to evaluate mJSW in healthy and osteoarthritic volunteers involves two parts; the fixed flexion X-ray positioning technique and the automated algorithm employed to quantify mJSW in the medial tibiofemoral compartment. In order to be able to use these techniques in research and clinical practice, the reproducibility of the technique must be assessed and shown to be satisfactory. Both short-term and long-term reproducibility were evaluated in this study by subjecting study participants to repeated X-rays. For each X-ray, the acquisition parameters including knee flexion, foot rotation and X-ray beam angulation were standardized and an automated algorithm to evaluate mJSW, requiring little user intervention, was implemented.

Over the short-term, the computer generated medial mJSW measurements yielded a RMSSD of 0.19 mm for all participants and a CV of 4.41%, figures which were slightly better than those obtained when user-intervention was involved (0.20 mm and 0.25 mm for the two readers). In separating "normal" participants from those with knee OA, it was determined that the reproducibility of healthy knees was better than for arthritic ones with a RMSSD and CV of 0.15 mm and 3.31% compared to 0.27 mm and 7.45%, respectively. Computer generated measurements of mJSW reliability revealed ICCs between 0.97 and 0.98 over the short

term, while those for the two readers were similar at 0.95-0.98. Over the long-term, reproducibility was assessed in healthy individuals with a computer-generated RMSSD of 0.14 mm, CV of 2.86% and an ICC of 0.99. These results suggest that mJSW measurements acquired from fixed-flexed radiographs and analyzed using an automated computer algorithm are highly reproducible over both the short-term and long-term.

Short-term reproducibility studies have been previously reported for fluoroscopic techniques as well as non fluoroscopic ones, using automated algorithms to quantify mJSW. For example, a study of short-term reproducibility employing the Lyon-Schuss positioning method and an automated JSW computer algorithm (Table 2) reported a CV of 3.5% in a small group (N=20) of OA and healthy knees (77). With the exception of the use of fluoroscopy, the Lyon-Schuss method is essentially similar to the fixed flexion method employed here. Similarly, in a large multicentre reproducibility study of 266 pairs of osteoarthritic knee X-rays using the semiflexed, fluoroscopic positioning technique and an automated algorithm to quantify mJSW, the RMSSD was found to be 0.12 mm and the CV was 3.18% (78). Another study of 25 osteoarthritic individuals X-rayed using the semi-flexed position with fluoroscopic guidance reported a CV of 5.5% (79). For non-fluoroscopic X-rays, a short-term reproducibility study conducted by Buckland-Wright reported a CV of 1.6% in a single-centre study of 74 patients with medial knee OA from radiographs

acquired in the MTP position (Table 2). However, this technique has been criticized because of the fact that the slight degree of knee flexion does not adequately capture the region of the knee most affected by articular cartilage thinning. This may contribute to the excellent reproducibility revealed in this study.

Short-term reproducibility studies conducted using the fixed flexion technique and automated computer algorithms have shown similar results. Among 30 knee OA patients who were X-rayed in the fixed-flexion position twice within a two week period, the CV of medial mJSW measurements was found to be 4.3% (80). In this study a positioning device known as the SynaFlexer (Synarc, Inc., San Francisco, CA) was used to improve the reproducibility of patient positioning. In a separate study of 28 healthy and knee OA patients who underwent a fixed-flexion knee X-ray where mJSW values were determined using the software algorithm employed in the current study, the RMSSD was reported to be 0.1 mm (42). The RMSSD was the same for X-rays acquired in the fluoroscopic semi-flexed position using the same automated algorithm.

Thus, in comparing short-term reproducibility data from the thesis with data from previously published studies, it is apparent that the reproducibility of the fixed-flexion positioning technique is comparable to that achieved from fluoroscopic radiographs and other studies of fixed-

flexion X-rays. To date, it appears as though this study is the first of its kind to report the long-term reproducibility of a technique used to acquire mJSW measurements. Our long-term reproducibility was similar to our short-term reproducibility and suggests that the fixed-flexion technique can be used over the long-term to evaluate mJSW in healthy individuals. This reproducibility is important in order to assess the smallest detectable difference in mJSW between two time points. Ravaud et al. discussed the means of determining statistically significant changes in mJSW over time (81). In an individual, the upper and lower cut-off points are $\pm 1.96\sqrt{2\sigma_\varepsilon^2}$ where 1.96 represents the 95% confidence interval (i.e. 95% confidence that differences are associated with a true change in mJSW) and $2\sigma_\varepsilon^2$ represents the variance of the difference between mJSW measurements at two time points (or ±1.96*SD(difference in mJSW)). Interpreting this would mean that change in mJSW lower than $-1.96\sqrt{2\sigma_\varepsilon^2}$ (individual lower cut-off or ICO_L) would represent a statistically significant worsening, a change greater than $+1.96\sqrt{2\sigma_\varepsilon^2}$ (individual upper cut-off or ICO_U) would represent significant improvement (increasing mJSW) and a change between these two values would represent stability over time (81). Ravaud et al. used this methodology to estimate the minimally relevant change in JSW in a sample of individuals with varying degrees of medial knee OA. Radiographs were acquired using fluoroscopy and joint space measurements were determined manually by two readers. The cut-off to determine a significantly relevant change in an individual was determined

to be 0.55 mm for one reader and 0.94 mm for the other reader (81). Using the 0.55 mm value and the annual rates of joint space narrowing that have been published (0.03-0.60 mm/year), it could take anywhere from 1 to 18 years to see such a difference. Using the 0.94 mm value, it could take 1.5 to 34 years to see such a difference. It is evident that achieving the smallest possible error in measurement is important in being able to detect small, yet significant changes in measurement over time thus leading to shorter time periods between examinations to observe these changes.

Having established satisfactory reproducibility, mJSW was evaluated in healthy and osteoarthritic individuals. The primary purpose of establishing normal ranges of mJSW in a healthy population of males and females is to provide "reference ranges" to which osteoarthritic values can be compared. In addition, it is important to determine if mJSW values appear to decrease in a healthy population or if, indeed, this is characteristic of only those affected by knee OA. Results from this study of healthy females of ages ranging from 20-69 years revealed that mJSW did not decrease with age but appeared, in fact, to remain constant with values ranging from 4.6 to 5.1 mm. The same trend appeared in males, although mJSW values were significantly larger in males compared to females with a normal range between 5.3 and 5.8 mm. When age is considered as a continuous variable, mJSW was related to neither age nor

BMI in either males or females. This suggests that joint space narrowing is not simply a consequence of aging as those in later decades appear to have mJSW measurements not significantly different from younger subjects. However, it is important to note that these data represent cross-sectional analyses and may differ from results of longitudinal follow-up of healthy patients. Thus, one should be cautioned about making definitive conclusions regarding a relatively constant measure of mJSW over time. In fact, one study conducted by Conrozier et al. reported changes in mJSW over time in patients without knee OA. Over a one year period, knees with K-L scores $\leq$ 1 acquired from Lyon-Schuss radiographs analyzed for mJSW using a software algorithm showed a mean (SD) rate of joint space narrowing of 0.05 (0.22) mm in those radiographs considered to have satisfactory metatarsophalangeal alignment (40). However, this study did not report whether or not this change was significant from baseline to one-year follow-up. In addition, it is important to note that this population without radiographic OA was a symptomatic population reported to be suffering from chronic (>3 months) knee pain who were referred to a rheumatology outpatient department (40). Thus, despite the absence of radiographic evidence of knee OA, it is apparent that these patients were not truly "healthy" because of their clinical presentation. Therefore, this estimation of "normal" joint space narrowing may not represent true changes in knee JSW in healthy individuals.

Despite the fact that there are few studies which are longitudinal in nature, there are a small number of cross-sectional studies which have investigated the relationship between JSW measurements and age. For example, a study of JSW values in 125 healthy individuals between 40 and 75 years of age acquired from standing, extended view radiographs and analyzed manually reported that mean medial JSW measurements did not decrease with increasing decade of life. In this study, males were generally found to have larger JSWs compared to females, with apparent mean JSW values of 5.0 to 5.5 mm compared to 4.5 to 5.0 mm, respectively (approximated from graphs) (82). This study also reported that BMI was not significantly related to JSW, results which are consistent with those reported in this thesis.

In contrast, a study conducted by Dacre et al. showed that joint space decreased with age group, although it was joint space area (mm^2) which was reported, not mJSW (83). Age groups, in this study, included 20 year intervals compared to 10 year intervals reported in previous studies. The study was also cross sectional in nature and had several methodological differences compared to those of this thesis and the one of Lanyon et al. (82). Participants included those who had reported to an Accident and Emergency Unit after minor trauma to the knees or with knee pain which required a knee X-ray. All X-rays were reported to be K-L grade 0, although patients were not free of symptoms and there was no

reported data on the history of the patients (i.e. previous history of knee trauma or injury, time of X-ray from trauma, bone or joint disease, etc.). X-rays for this study were acquired in a non-weight bearing position and a digital image system was used to analyze an area of the medial joint space 1 cm in size while the width of the tibial plateau was measured manually using a ruler (83). Despite the fact that all radiographs were considered radiologically "normal", the differences in the way the X-rays were acquired and analyzed, the symptomatic nature of the population, the lack of distinction between males and females and the fact that the sample included a large number of young men who had experienced knee trauma means it is unreasonable to compare results with the Dacre et al. study.

Yet another study investigating the relationship between age and joint space in healthy individuals reported a decrease in mean joint space size with increasing age. Conducted by Sargon, et al., this study used coronal images from magnetic resonance images to analyze joint space in 184 patients who were assessed as having "normal" knees on MR. In this case, the medial knee joint space was measured from the most medial part of the knee, an area which includes cortical bone, articular cartilage, menisci and joint fluid (84). With each decade, the mean joint space width in the medial compartment decreased from 7.07 (0.29) mm at 21-30 years to 5.24 (0.18) mm at ≥ 61 years. Again, however, this study differed in

many ways from this thesis (84). For instance, images were acquired in a non weight-bearing position with measurements being made at the level of the cruciate ligaments although the way in which the joint space was measured was not reported. In addition, the number of males and females in the population and in each age group was not reported and it was not known why these study participants were referred for a knee MRI (i.e. were they symptomatic?). The justification for measuring mean joint space width at the most medial point of the knee was not given and leads one to question the clinical use of this measurement as this is not typically the region of joint space narrowing in osteoarthritic individuals. In addition, narrowing which does occur at this region of the knee may take place because of loss of fluid, damage to the meniscus, etc., and not due to thinning of cartilage as this is not a primary weight-bearing area. The methodological differences may explain why a decrease in joint space was found with age in this population compared to the results reported in this thesis.

Other studies of medial JSW values in healthy individuals have reported average values for the entire populations under investigation but have not analyzed these measurements as they varied with age or sex. In this thesis, the mean (SD) mJSW in 73 healthy females is 4.82 (0.70) mm and 5.59 (0.66) mm in 45 healthy males. An estimate of mean JSW in a control population of 10 individuals in the medial compartment of the knee

was 3.8 ± 0.3 mm acquired from Lyon-Schuss positioned X-rays and an automated algorithm for mJSW measurement (77). Details of the control population (i.e. mean age, number of males and females) were not reported. While Sargon (84) reported a mean joint space width acquired from the analysis of MR images in "healthy" knees to be 5.68 (0.27) mm for females and 6.81 (0.32) mm for males, Dacre et al. (83) reported the mean medial joint space width acquired from weight-bearing radiographs in a subgroup of 30 radiologically normal knees to be 5.15 (0.30) mm. In separating males from females, the mean medial joint space acquired from non weight-bearing X-rays was 5.73 (0.15) mm in females and 7.03 (0.12) mm in males (83). However, it is generally accepted that joint space width values acquired from non weight-bearing X-rays are larger than those from weight-bearing ones, suggesting that these results may, indeed, be consistent with those reported in this thesis.

Unfortunately, there is a paucity of data reporting longitudinal joint space narrowing results in healthy individuals leading researchers to extrapolate data from cross-sectional analyses. While some studies suggest that joint space width decreases consistently with age in healthy individuals, others, such as the one described here, have shown that those who are older do not have significantly smaller JSW values than those who are younger suggesting that JSW remains constant with time. Differences in the definition of a "normal" or "healthy" population (i.e.

radiographically vs. symptomatic) and the differences in X-ray acquisition and analyses techniques likely account for the disparity in results. However, the use of a reproducible, weight-bearing X-ray acquisition technique and an automated analysis algorithm implemented to study a radiographically and symptomatically "normal" population likely provides the most accurate results regarding mJSW across the ages. To date, this study is the only one of its kind. Ranges of mJSW for healthy individuals do now exist with men having slightly larger spacing than women. This study also suggests that joint space narrowing does not appear to be a result of the normal aging process.

Identifying ranges of mJSW measurements for osteoarthritic individuals were difficult to perform for a few reasons. In order to estimate "OA" ranges of mJSW in different age groups, it is necessary to collect data from those with knee OA who are of all ages. A problem arises because it is difficult to recruit osteoarthritic volunteers who are under the age of 40 years, primarily because the disease seems to be associated with increasing age. In addition, there is also difficulty in recruiting patients over the age of 60 years who meet the inclusion and exclusion criteria such as those laid out in this study (i.e. no diabetes, no bone or joint disease, no knee surgery, etc.). Because of these challenges there was a wide variation in disease severity across age groups and small numbers of individuals in the younger and older groups as shown in Table

7 and Figure 15. However, the objective, quantifiable nature of mJSW makes it possible to compare values in those with knee OA to those who are "healthy". From Table 7, it is apparent that osteoarthritic females who are 40-49 years of age have a mean mJSW which is approximately 4.5 mm and those 50-59 years of age have a mean mJSW of 4.2 mm. These mJSW values are slightly less than the "normal" range for healthy females of 4.6-5.1 mm. Likewise, the mean value for osteoarthritic males ≥ 40 years was < 4.5 mm, a value smaller than the "normal" range identified in healthy males of 5.3 to 5.8 mm. These results show that individuals with knee OA indeed have smaller medial mJSW values than healthy individuals and that there may be a justification for establishing "cut-off" points below which people over the age of 40 years may be identified as having knee OA. For females, this value lies between 4.0 and 4.5 mm while for males it lies between 4.8 and 5.3 mm. A study conducted by Buckland-Wright et al. also reported mean mJSW values as they varied with age group in a clinical osteoarthritic population that was radiographed in a semiflexed fluoroscopic view and analyzed for mJSW using a computer algorithm (78). Although these values were not separated by sex, a decrease in mean mJSW was observed per decade. For instance, those < 50 years old had a mean (95% confidence interval) mJSW of 4.2 (3.9-4.5) mm while those between 51-60 years of age had a mean mJSW value of 3.9 (3.6-4.2) mm, those between 61-70 years of age had a mean mJSW value of 3.6 (3.3-3.8) mm and those ≥ 70 years had a mean mJSW

value of 3.4 (3.2-3.7) mm (78). These ranges agree with those revealed by this study and are consistent with the cut-off points proposed. Thus, for the purposes of establishing cut-off points, it may be reasonable to suggest that those individuals who are younger than 40 or even 50 years of age and have a mJSW value smaller than the lower limit of the 95% confidence interval of the age group may have early radiographic OA. Such cut-off points may be useful in selecting patients who may be eligible for studies of disease modifying pharmacological agents. For instance, these values could be implemented in establishing inclusion/exclusion criteria where those with mJSW measurements greater than the cut-off point would not be eligible to participate because they still have "normal" mJSW values and only those with smaller values would meet the inclusion criteria.

Despite the potential applicability of cut-off points, the wide variation in mJSW shown by large confidence intervals in each group may not reveal the actual relationship between age and mJSW in people with knee OA. In addition, given that there are varying stages of disease severity in each age group, the data are likely more meaningful when mJSW is presented as a function of age as a continuous variable or as a function of disease severity. Both of these relationships were explored.

To investigate the relationship between mJSW and aging in osteoarthritic patients, regression analyses were performed. Results revealed that age is a significant predictor of mJSW as was shown by a β value of -0.431 for males and -0.479 for females. This suggests that mJSW decreases with increasing age in patients with knee OA. In females with OA, increasing BMI was also significantly related to decreasing mJSW as shown by a β value of -0.349. Once again, however, the issue of cross-sectional results arises. In order to definitively determine that mJSW decreases with age, longitudinal data should be collected. Such studies have been conducted and have reported rates of joint space narrowing. However, results have varied extensively with published rates of loss ranging from 0.03 to 0.60 mm/year (40;85). Such discrepancies lead one to question the overall quality of the studies with special attention paid to the population being studied and the methodology that was employed to yield the results. In addition, different study durations and sample sizes make comparisons between studies difficult as commented in a review by Mazzuca et al. (85). This review also suggested that rates of joint space narrowing published in population-based studies are slower (i.e. 0.06-0.10 mm/year) than those from clinical samples (generally ≥ 0.2 mm/year). Two more recently published studies conducted over a two year period reported significant decreases of 0.24 ± 0.50 mm (N=58) and 0.13 mm (CI = 0.008-0.245 mm) (N=42) over the study duration (86;87). A third study conducted over a one year period

reported a mJSW decrease of 0.34 ± 0.50 mm (N=73) (40). All three studies acquired fluoroscopically guided X-rays, two of which used computer techniques to analyze mJSW in clinical populations (40;87) while the third used manual methods in a population-based sample with chronic knee pain (86). Unfortunately, stages of disease severity were not reported in these analyses so the issue of dissimilar sample populations remains. Despite this, these results do suggest that changes in mJSW can be detected and quantified over time. Thus, these results and those of previously published studies suggest that joint space narrowing does not occur at a constant rate but accelerates with changes with severity (85;88).

An investigation of the relationship between disease severity and mJSW was performed in this thesis and it was evident that mJSW decreased significantly with increasing K-L grade as shown in Figure 16. Those with mild knee OA (K-L=2) had significantly larger mJSW values than those with moderate (K-L = 3) and severe (K-L grade 4) disease. Semi-quantitative scoring systems other than the K-L system have been used to evaluate knee OA, specifically scales referring to only joint space narrowing. In fact, studies which do not quantify mJSW often report joint space narrowing scores or use both in combination (77;78;82;89). A study performed by Piperno et al. reported the relationship between mean joint space and varying degrees of joint space narrowing as defined by an

original scale (77). Like the results here between K-L grading and mJSW values, there was no difference in mean JSW identified between those with scores of 0 (no joint space narrowing) and 1 (doubtful narrowing). The introduction of a subjective judgment in the joint space narrowing scoring system presents similar problems to those in the K-L system discussed previously. However, studies such as these provide evidence that semi-quantitative scoring systems do have a place in evaluating knee OA severity, although the lack of exact, objective measures leaves room for subjectivity and variability in grading.

While being able to evaluate radiographic evidence of disease presence and severity is extremely important both in clinical and research practices of knee OA, the criteria by which most patients measure disease presence and severity is by symptoms, specifically pain, stiffness and function (90). For example, in measuring changes in osteoarthritic features over time, it is much more meaningful for a patient to identify increases in morning stiffness or pain during stair climbing than to be able to relate to a decrease of 0.25 mm in minimum joint space in the medial tibiofemoral compartment. However, due to the subjectivity in symptom reporting and the bias that is introduced, objective methods of evaluating the state or stage of OA are also important in order to accurately and sensitively measure disease progression and to evaluate the effectiveness and efficiency of potential disease modifying agents. Thus, there has

been great interest in exploring the relationship between radiographic features of knee OA and the clinical symptoms experienced by those with the disease.

Numerous studies have been conducted to investigate such relationships. To date, the vast majority have evaluated the presence and severity of radiographic features of OA, such as osteophytes and joint space narrowing, using semi-quantitative scoring techniques such as the K-L grading system. Symptomatic data has been collected primarily by through the use of the WOMAC questionnaire and by asking very simple questions specifically regarding pain. Unfortunately, results of these studies have been mixed; some suggest these variables are related to one another while others found no such associations (91-94). Reasons for these discrepancies have been attributed to the differences in study populations (i.e. population based vs. clinical vs. those awaiting elective knee arthroplasty), sizes of study populations and methodology (outcome measures of pain on WOMAC vs. "ever" pain). However, until very recently, these studies had only employed semi-quantitative scoring systems to evaluate radiological features associated with knee OA and had not used objective, quantifiable measures such as mJSW. To my knowledge, the first and only study to explore the relationship between mJSW and pain was published by Bruyere et al. in 2002 (95). A randomized, placebo controlled clinical trial designed to test the effects of

1500 mg/day glucosamine sulphate on mean joint space width (primary outcome) and clinical symptoms, this study included 212 patients > 50 years old with diagnosed knee OA (ACR criteria, all K-L grade 2 or 3) (96). Radiographs were acquired with the knees fully extended (with fluoroscopy) and mJSW was analyzed manually using a magnifying lens. At baseline, no significant correlations were identified between mJSW and the total WOMAC score (r=-0.07, p=0.3), the pain subscale (r=-0.08, p=0.27), the stiffness subscale (r=-0.05, p=0.51) or the function subscale (r=-0.07, p=0.35). In contrast to these results, the thesis revealed significant correlations between the total WOMAC score and all subscales in both males and females as revealed in Table 8. Discrepancies between the results of the two studies can possibly be explained by the differences in techniques used to measure mJSW. In the thesis, participants were positioned with knees in fixed-flexion (approximately 20°) and mJSW was obtained from a validated automated computer algorithm. It has been shown that the fixed-flexion positioning technique is more reflective of cartilage degeneration because the location of the femur where thinning occurs is captured at this angle of knee flexion but is not when the knee is in full extension. Thus, radiographs acquired in full extension may not accurately reflect what is actually occurring in the joint. In addition, the study population under investigation here included those with K-L grades 0 to 4, compared to the Bruyere study which included only those with grades 2 and 3. It is possible that there was not a wide enough range of

mJSW measurements within these two grades to be reflective of differing symptoms, compared to this thesis which had a wide range of disease severity and symptomatology. With improved methodology of the study performed here compared to that of Bruyere, specifically the use of the fixed-flexion technique of X-ray acquisition and the automated analysis of mJSW, the results suggest that, indeed, mJSW measurements are significantly related to pain and stiffness in the knee and to decreased functional capability associated with knee OA.

Results from minimum joint space width analyses performed here suggest that the measurements are valuable not only because they can objectively and reliably demonstrate structural changes in articular cartilage over time, a major feature of knee OA, but because they correlate significantly with pain, stiffness and functional symptoms experienced by the patient. However, recent research in knee OA has shown that cartilage is not the only tissue affected by the disease. Bone, specifically subchondral bone adjacent to the cartilage, also plays a significant role in disease pathophysiology. Thus, we felt it was important to evaluate bone tissue in these regions by performing DXA scanning around the joint, to evaluate bone mineral density in the distal femur and proximal tibia. In order to determine the effect of knee OA on subchondral bone, an investigation of BMD in healthy individuals was first conducted and used as a reference for comparison. In addition, BMD in the L-spine

and proximal femur were also analyzed in healthy males and females and compared to those with knee OA. Previous studies have suggested that those with knee OA have higher BMD values in these regions than those without (97-99), and this thesis was able to test this hypothesis.

Bone mineral density in the L-spine and hip was not significantly related to age in this study population suggesting that BMD does not decrease with normal aging in healthy males and females. However, many large scale population based studies have been conducted to investigate BMD and its relation to aging and have shown that BMD in the L-spine and hip in fact does decrease with age (100-102). Possible reasons for the lack of significance shown in this population are the size of the sample, the cross-sectional nature of data collection and the fact that the study population was not representative of the population at large. Generally speaking, decreases in BMD which have been identified are quite small and bone loss often does not begin until the fifth decade of life. The fact that there were few "healthy" participants over the age of 60 years combined with the fact that study population was different from those of the studies mentioned above likely explain why these results did not show decreases in BMD with age.

Similarly, results in the regions of the distal femur and the proximal tibia showed that BMD was not significantly related to age as was the

case in a study reported by Yamada et al (103). In healthy females, mean BMD in the subchondral region of the femur and in the total distal femur was 0.939 and 1.007 g/cm^2, respectively, and in the subchondral region of the tibia and in the total proximal tibia, was 0.897 and 0.943 g/cm^2, respectively. Results of the tibial analyses revealed that mean values were consistent with those in healthy females of similar ages yielded by the study by Checovich et al. (50). In healthy males, BMD was similar with mean values of 0.938 and 1.119 g/cm^2 in the subchondral region of the femur and in the total distal femur and 0.958 and 1.044 g/cm^2 in the subchondral region of the tibia and in the total proximal tibia, respectively. Once again, these values were similar to those yielded from an additional study where the mean (SD) tibial BMD of 0.956 (0.174) g/cm^2 in healthy males and females was comparable to that found in this study (59). To my knowledge, "normal" values of distal femoral BMD have never been published. However, the relationship between tibial BMD and age derived from this study is not consistent with that previously published. For instance, the study conducted by Checovich et al. in 44 healthy women revealed that BMD in the proximal tibia decreases significantly with age (50). Mean (SD) values of proximal tibial BMD in women 20-49 years old were consistent with those given in this thesis, although significant decreases in BMD were seen to occur after the age of 60 years (50). It should be noted, however, that only 10 females in the Checovich study were ≤ 49 years of age with only one female between 40-49 years of age

leading one to question if enough study participants were included in order to make such conclusions. In addition, Checovich also reported that 14 BMD analyses revealed individual spine measurements with vertebral pathologies (such as 3 crush fractures) suggesting that this study population was not entirely "healthy" to begin with but that a number of participants had osteopenia or osteoporosis. Thus, decreases in tibial BMD with age may be attributable to bone disease rather than simply aging (50). Similarly, Bohr and Schaadt showed decreases in tibial BMD with aging in a "normal" population of females ($r=-0.72$) at a rate of 8-9% per decade (57). Also in females, height and weight were found to be positively correlated with BMD in females as was the case in this thesis. However, no such significant relationships between age, height and weight and tibial BMD were found in males as was also the case reported here. Unfortunately, "normal" in this study population was not well defined and it is not known whether those defined as such had "normal" BMD measurements in the L-spine and hip. Once again, factors likely accounting for discrepancies in results are the differences in methods used to acquire and analyze tibial BMD measurements and sample populations.

With two studies reporting results contrary to those given here, the question of whether the techniques used to evaluate subchondral BMD in this study are sufficiently precise and reproducible to achieve accurate

results arose. In fact, weaknesses and limitations associated with the scanning and analysis methodology implemented in this protocol became apparent during the data analysis phase. The most evident limitation appeared when scanning knees that were malaligned, in most cases in a varus deformity with medial tibiofemoral compartment narrowing as shown in Figure 44. The technique was first developed and validated in individuals with spinal cord injuries and it is unknown whether varus deformities were present in this population. Ideally, the femur and the tibia in these scans are aligned parallel to the sides of the X-ray table, as shown in Figures 8 and 9. Unfortunately, even in the "healthy" study population, a seemingly large proportion of individuals appeared to have some degree of varus malalignment. In the OA population, this proportion was even greater with some individuals showing severe malalignment such as that displayed in Figures 45 and 46. The relationship between varus malalignment and knee OA has been extensively studied (104-106). What is obvious is these DXA scans are the differing amounts of medial and lateral subchondral bone included in the subarticular regions of the analyses, specifically regions F4 and T1. For example, in those with narrowing of the medial compartment, the majority of bone analyzed in the femur is in the medial compartment with very little contribution from the lateral compartment compared to the tibia where the opposite is the case. In individuals with varying degrees of varus alignment, these proportions change making it very difficult to compare BMD values between

individuals. At the time of the initial protocol design for the analysis of distal femoral and proximal tibial, the DXA machine that was used was the Hologic 4500A, an older machine than the newer Hologic Delphi scanner used in this thesis. The original protocol did not allow for compensation to be made in angling the lines such that they were parallel to the tibial plateau, and thus no adjustments were made here. In addition, measurements of the femorotibial angle were not collected and, thus, results did not account for this malalignment. Other studies such as those by Hulet et al. and Wada et al. have performed such analyses and determined that BMD is actually higher in the medial tibial compartment than the lateral compartment (59;60;107). Also accompanying the problem of the malalignment and varying amounts of bone in each compartment of the most distal part of the femur and proximal part of the tibia was the involvement of the fibula. The overlap between the lateral tibia and the head of the fibula was inconsistent between participants and this could also have led to variations in results as a larger area of overlap would likely increase the value of BMD because of overlying bone on bone compared to very little overlap. By changing the area of bone analyzed for BMD it is possible that this area of overlap could be eliminated.

In osteoarthritic individuals, two additional problems arose which are evident in Figures 45 and 46. The physical presentation of osteophytes, specifically on the tibial spines and on the outer margins of

the tibia and femur presented difficulties in analyzing the DXA scans. The matter of whether or not to include the osteophytes in the shaded area of bone for BMD calculation was the first issue and, if they were not to be included, defining the border between the "normal" bone and the osteophyte was the second. Because of this challenge, all osteophytes were included in the shaded bone area of interest. However, this presents intuitive problems of the accuracy of osteoarthritic BMD since the addition of osteophytes might alter true BMD measurements as has been shown in previous studies where the presence of spinal osteophytes had a significant impact on BMD in the vertebral column (108;109). The second challenge which arose occurred in the analyses of BMD in those with moderate to severe knee OA where the distal femur came into near or direct contact with the proximal tibia. In such cases, defining the outer margins of the tibial plateau and the femoral condyle was difficult and likely introduced an element of error to the measurements as these borders were drawn manually and were not objectively determined software.

Despite these potential weaknesses, BMD in the L-spine, proximal tibia, distal femur and proximal tibia was analyzed in the osteoarthritic study population. In females with knee OA, both age and BMI were significantly related to BMD in the spine and hip, although the same was generally not true for males. Perhaps more importantly was the finding

that BMD in these regions was not significantly different between those with and without OA. The majority of studies which have investigated the relationships between OA and BMD, however, appear to suggest results to the contrary, although the reasons for these observations remain largely unknown (97-99;110-115). If, in fact, lumbar spine and hip BMD are higher in those with knee OA, it is possible that this study was underpowered to detect such differences. In addition, there may not have been enough individuals with moderate to severe knee OA to reveal differences in BMD. In fact, calculations of power revealed that this was indeed the case as the samples were not adequate to be able to detect differences in the BMD at the L-spine and hip between healthy and osteoarthritic males and females. In comparing healthy and osteoarthritic females, there was 58% and 38% power to detect differences in L-spine and hip BMD, respectively. To achieve a power of 80% ($\alpha = 0.05$), 142 females would be needed to detect such differences in L-spine BMD while 149 individuals would be required to detect such differences at the total hip. Likewise in males, there was only 25% and 14% power to detect differences in L-spine and hip BMD between those with and without knee OA, respectively. Again, to achieve a power of 80% ($\alpha = 0.05$), 240 males would be required to detect differences between healthy and osteoarthritic males at the L-spine compared to 510 individuals required for differences in BMD to be detected at the total hip. It should also be noted that osteoarthritic individuals with other bone or joint diseases (i.e. osteopenia)

were excluded from the study therefore leaving only individuals with "normal" BMD values in the study population giving rise to a somewhat biased sample. This was not the case with other published studies where many were population based samples which would have included a wide range of BMD measurements.

Bone mineral density analyses in the distal femur and proximal tibia paralleled those in the L-spine and hip where no significant differences were observed between the two study groups (OA vs. "healthy"). To my knowledge similar analyses have not been conducted. Due to uncertainties in measurement techniques as previously described, one cannot be certain that differences in BMD do not actually exist. For instance, varus alignment was both more common and more severe in OA patients thus causing more of the medial femoral condyle to be included in the F4 region of the BMD analyses in those with OA than in healthy males and females. Although these results would have altered BMD values in the subchondral regions, total BMD values (average of the 4 regions) would have been affected only to a very minor degree since the subchondral regions account for only 20% of the total area under investigation. This would make comparisons of BMD between these two groups of individuals difficult because proportions of bone in the analyses are not the same. This has been shown in studies where comparisons of

medial compartment BMD to lateral compartment BMD revealed a higher density in the medial side of varus deformed knee OA patients (59;107).

Although no significant differences between BMD in the distal femur and proximal tibia were detected in those with diseased knees compared to those without, significant relationships between age and BMI and BMD in these regions were identified in the female population. Age was found to be negatively related to BMD (β-coefficients of -0.520 to -0.620) suggesting that BMD decreases with aging in an osteoarthritic population. This observation was not seen by Wada et al. in a study of tibial BMD in osteoarthritic individuals (107). Results showed that medial tibial BMD was significantly lower in a group of younger individuals than in a group of older individuals also with knee OA with mean values of 0.67 g/cm^2 compared to 0.77 g/cm^2. However, other significant differences including a higher BMI and increased disease severity existed between the two groups, and such differences were not accounted for in the analyses (107). Similarly, the way in which the results were presented also made it difficult to claim that those with more extensive disease (K-L grades 3 and 4) have higher medial tibial BMD than those with more mild disease (K-L grades 1 and 2) because differences in age and BMI were not taken into account. However, the relationships between disease severity and BMD in the distal femur and proximal tibia were investigated in this thesis. As displayed in Figures 27 and 28, increasing disease severity was

significantly associated with increased BMD in the distal femur and proximal tibia as supported by correlation coefficients of 0.352 and 0.284, respectively. However, increasing disease severity is also associated with greater varus deformity in the knee (bone on bone contact) and this data was not accounted for in the analyses. Thus, despite the fact that there is a positive, significant relationship, the results do not conclusively suggest that increasing K-L grade or disease severity is the cause of high BMD values.

Not only was age significantly related to BMD in the subchondral region of the femur and the total distal femur, so too was BMI. In fact, BMI significantly predicted BMD in these regions (F4 and T1) with β-coefficients of 0.407 and 0.389, respectively. In the tibia, the relationships were stronger with corresponding β-coefficients of 0.561 and 0.629, respectively. This may be explained by Wolff's law which states that bone architecture at a specific location is dictated by the mechanical stresses applied to the bone (116). This law has been demonstrated in previous studies and is also supported by results showing that BMD is higher in the medial rather than the lateral compartment in those with medial knee OA (59;107;117-119).

Unfortunately, as discussed earlier, limitations and inconsistencies in methods implemented in this study decrease the value of the results

and provide uncertainties as to whether the same methods should be employed in other studies. Thus, the analysis of subchondral bone in those with knee OA may be better studied using magnetic resonance imaging technology. While this area of research is still in its very early stages, it is possible that the advantages of pQCT and possibly also MRI over DEXA may yield information about the role of subchondral bone in OA that cannot be identified using simpler technology. In fact, to date, advances in MR imaging and post-image analyses have provided insights into osteoarthritic changes that occur in articular cartilage that extend what was initially learned using plain radiography. Assessments of articular cartilage volume and thickness allow a number of fundamental and clinical research issues to be addressed. Such issues include factors which affect the incidence and progression of cartilage degeneration, rates of change in osteoarthritic individuals and the ability to evaluate responses to therapeutic interventions. In this study, normal ranges of cartilage volume and thickness were assessed in males and females of various ages and compared with those obtained from osteoarthritic individuals.

Just as with mJSW measurements, inter-observer variability was determined for measurements of cartilage volume and thickness in a sub-group of study participants. Composed primarily of knee OA subjects, 28 images were analyzed by two independent readers and results were compared. The intra-class correlation coefficient for medial tibial cartilage

volume was 0.772 and 0.767 for medial tibial cartilage thickness. Likewise, coefficients of variation for medial tibial cartilage volume and thickness were 17.4% and 17.1%, respectively. Unfortunately, these values show less agreement between readers than those that have previously been published. For example, in a study of the inter-rater reliability performed on 10 healthy and 18 osteoarthritic individuals, ICC values ranged from 0.943 to 0.988 for all three readers in different regions of the knee (120). Just as in the current study, agreement between readers was lower (0.943) when just the osteoarthritic subjects were involved in the analyses compared to just the healthy individuals (0.988). Similarly, inter-rater reliability measures repeated in previous studies have shown CVs of 6.6% for cartilage thickness and 5.5% for cartilage volume when using a rule-based protocol (121). However, estimates of inter-rater agreement of medial tibial cartilage volume have also been relatively close to those observed here. Lindsey et al. reported CVs for inter-observer reproducibility, including normal and osteoarthritic patients, of 13.0% for tibial thickness and 25.5% for tibial cartilage volume from images acquired in the sagittal plane with a slice-thickness of 2.0 mm (122). Such was also the case for estimates of inter-reader agreement reported by Gandy et al. and Stammberger et al. where the CVs for medial tibial cartilage volume were reported to be 14% and 11.4%, respectively (66;123). In the latter study, 7 technicians segmented 15 "healthy" data sets both manually and using B-spline snakes acquired in the coronal and sagittal planes.

Coefficients of variation for medial tibial cartilage volume and maximal cartilage thickness were 8.3% and 9.0% using the manual technique and 7.8% and 6.0% using the snake technique (66). For images acquired in the sagittal plane, CVs for medial tibial cartilage volume and maximal cartilage thickness were 11.4% and 11.7% using the manual technique and 10.8% and 6.1% using the snake technique. Evidently, the manual technique of cartilage analysis revealed greater variability in measurement than the snake technique. Images acquired in the sagittal plane were also seen to be less reproducible than those acquired in the coronal plane (66). Both features, sagittal acquisition and manual segmentation, were employed in this study. In addition, only 5 of 28 images included in this reproducibility study were considered "healthy". Since it is well known that the analysis of osteoarthritic images is less reproducible than healthy ones, the combination of these three factors may explain the high variability in measurements in the current study. Unfortunately, inter-rater reliability measures were higher in this study than in those previously published leading one to question whether segmentation training was sufficient. Segmentation training for one reader occurred during a one month period where the majority of training focused on the segmentation of "healthy" images which, by nature, are simpler to segment due to the absence of cartilage defects. In addition, no training software was available at the time and, thus, training was conducted primarily by an experienced reader who was not fluent in English. Another potential

cause of the poor inter-rater reliability obtained here was the nature of manual segmentation. The term "manual" suggests that there is the potential for subjectivity to be introduced into each slice when considering where cartilage "starts" and "stops", and which image slice segmentation should begin and end. Variations between readers in these two particular aspects of manual segmentation likely accounted for the difference measured in this study.

In multi-centre or longitudinal studies of knee OA where more than one reader may be segmenting MR images, it is extremely important to achieve high inter-rater reliability to avoid the possibility of inconsistencies in results or the likelihood that observed differences between individuals or groups are a result of discrepancies in measurement between readers. Greater variability in measurements mean that larger sample populations are required to conduct studies which, in turn, leads to increase costs. In this thesis, all images were analyzed by a single reader. Thus, despite the inferior inter-rater reliability results of this study, comparisons of cartilage volume and thickness can still be made between individuals and groups. In healthy females, for example, the average (SD) medial tibial cartilage volume was 1.53 (0.26) mL while in healthy males, the average (SD) was 2.33 (0.47) mL. The average (SD) medial tibial cartilage thickness was 1.45 (0.18) mm for the females and 1.72 (0.24) mm for the males. In both cases, males had significantly larger medial tibial volume and thickness

than females (also shown in Figures 32 and 33). These results lie consistently in the range of those previously published. For example, in a study of 57 healthy postmenopausal women, Wluka et al. (124) found the mean medial tibial cartilage volume to be approximately 1.53 (0.31) mL while Faber et al. (125) found the mean medial tibial cartilage volume to be 1.31 (0.29) mL in a sample of younger, healthy females. Similar results have been published in healthy males where medial tibial cartilage means of 1.92 (0.49) mL and 1.59 (0.32) mL have been reported in young individuals as have values of 2.63 (0.50) mL in a slightly older healthy male population (<50 years) (125-127). With respect to cartilage thickness, mean values for young healthy women have been reported to be 1.20 (0.19) mm while for young healthy males, the average was reported at 1.36 (0.15) mm (125). In this latter case, the difference between males and females was not statistically significant. In a cross-sectional study of 372 healthy individuals <45 years old, the mean thickness was found to be larger at 3.8 (0.7) mm (128).

The issue of whether sex, age and other variable such as height, weight and BMI are significantly related to cartilage volume and thickness has been the subject of many studies. In the current study, results showed that males have significantly larger medial tibial cartilage volume and thickness than females. Previously published studies have shown mixed results with respect to differences in volume and thickness between males

and females. For instance, Cicuttini et al. reported that, independent of BMI and bone size, both medial tibial cartilage volume and thickness were greater in males than in females in a group of 166 healthy individuals aged 21-79 years (129). Similarly, in a study by Faber et al., healthy young males were found to have significantly larger medial tibial cartilage volume compared to females, but no such significant differences occurred between medial tibial cartilage thickness (125). However, differences in medial tibial cartilage volume between males and females were not significant after results were normalized to body weight or body weight x body height. Differences in cartilage thickness remained insignificant after adjusting for weight and weight x height (125). The same was true in a study conducted by Cicuttini et al. where initial differences in tibial cartilage volume between males and females became non-significant after adjusting for body and bone size in a group of radiographically healthy, yet symptomatic males and females (N=28) (130). However, in a cross-sectional study of 372 healthy males and females, significant differences between medial tibial cartilage volume existed even after adjusting for body height, weight and bone size (127). Discrepancies in results from these studies likely exist because of differences in sample populations (i.e. age, definition of "healthy"). The current study did not adjust for differences in bone size, and by doing so, it is plausible that significant differences in cartilage volume and thickness between sexes that were observed may be eliminated to agree with previously published results.

In the current study, results also showed that age and BMI were significantly related to medial tibial cartilage volume but that only age was related to thickness in healthy females. In other words, increasing age was associated with decreasing cartilage volume (β=-0.407) and decreasing thickness (β=-0.374) after adjusting for BMI. In contrast, neither age nor BMI were found to be significantly related to either medial tibial volume or thickness in males. Other studies which have investigated the relationship between age and cartilage volume and thickness have shown inconsistent results. For instance, Ding et al. showed that age correlated negatively with medial tibial cartilage thickness (β=-0.014), but not volume, after adjusting for sex, height, weight and bone size in a large cross sectional study (128). However, after separating males from females, Pearson correlation coefficients showed that age and medial tibial cartilage thickness were significantly related in males, but not females (r=-0.20). Once again, similar correlation analyses showed no such significant relationships existed in males or females (128). In contrast, a study of 45 healthy Caucasian males (mean age = 52.5 years) showed that both age and BMI were significantly inversely related to medial tibial cartilage volume with regression coefficients of -0.01 and -0.05, respectively (131).

Two other studies which also examined the effect of age on cartilage volume and thickness did so by comparing "younger" groups to "older" groups. Using 45 years of age as the division between the two groups of healthy individuals, significant differences were identified in medial tibial cartilage thickness as shown by a mean thickness of 3.8 (0.7) mm in the younger group compared to 3.6 (0.7) mm in the older group (128). Significant differences in medial tibial cartilage volume were not observed. These results are consistent with those published by Hudelmaier et al. who compared medial tibial cartilage thickness in younger (mean age approximately 25 years) with older (mean age 60-61 years) males and females (126). Once again, no significant differences were identified between younger and older females and younger and older males, suggesting that cartilage thickness does not decrease with age. However, one should be cautious about the interpretation of these results as both studies were cross sectional in nature and did not follow individuals longitudinally. Two studies of healthy individuals which have reported longitudinal changes in cartilage volume over time have shown that cartilage volume does, indeed, decrease with aging. In healthy males (N=28, mean age 52 years), the mean annual reduction in tibial cartilage volume was found to be 2.8% (95% CI = 0.2% to 5.5%) (132). In healthy postmenopausal females, the average annual decrease in total tibial cartilage volume was similar at 2.4% (3.2%) (124) while the annual reduction in the medial tibial compartment, specifically, was found to be

2.4% (3.6%). What is notable in these two studies is the age of inclusion of subjects who are being investigated for cartilage loss. In both cases, the mean age of participants was over 50 years. It is beyond this age that small decreases in cartilage volume have been observed. To this point, longitudinal studies of changes in cartilage parameters have not been studied in those younger than mid-age. The current study suggests that there is a linear decrease in medial tibial cartilage volume and thickness with age in healthy females and this decrease appears to start earlier than the 6^{th} decade, although, once again, it should be noted that these data are cross-sectional in nature. Despite this, the small decrease in cartilage volume that has previously been observed appears consistent with the results of the current study as shown by a small decline over the age groups for females as depicted in Figure 32. It is important to note, however, that no significant differences in medial tibial cartilage volume were detected between age groups. Changes in cartilage thickness over time have not yet been reported, although Figure 33 would suggest a similar trend. Such declines were not observed in males in the current study, although the small sample size and the relatively small number of healthy males over 50 years of age may help to explain this inconsistency.

Although few studies have reported the influence of height, weight and BMI on cartilage volume and thickness in healthy individuals, most studies adjust for these measures when examining the effects of age on

volume and thickness. Two studies have revealed significant correlations between these variables. For instance, body weight was found to be significantly related to medial tibial cartilage volume, but not thickness, in healthy young males and females (r=0.61) as was the interaction between body weight and height (r=0.61) (125). Likewise, BMI was found to be significantly related to medial tibial cartilage volume in healthy men as shown by a regression coefficient of -0.05 (131).

While there is still some degree of disagreement as to whether age is significantly related to cartilage volume and thickness in healthy individuals, there is little doubt that there is a negative association with age for these variables in those diagnosed with knee OA. Regression analyses performed in the thesis revealed that age was, indeed, significantly negatively related to medial tibial cartilage volume and thickness in both males and females with regression coefficients ranging from -0.430 to -0.480. In females, BMI was also significantly negatively related to volume and thickness as shown by β-coefficients of -0.398 and -0.401, respectively. Other studies which have detected similar relationships have been longitudinal in nature, typically lasting approximately two years. For example, changes in medial tibial cartilage volume over the study duration were evaluated in a study of 123 osteoarthritic individuals (mean age (SD) approximately 63 (10) years), the majority of whom had mild-moderate disease as assessed by plain

radiographs (133). In males, the average (SD) annual decrease in medial tibial cartilage was 5.1% (6.5%) while in females the decrease was 4.5% (6.5%). In spite of the fact that the current study was cross sectional in nature, both studies revealed that there were no significant differences in annual medial tibial cartilage loss between males and females. Thus, the average annual rate of loss of medial tibial cartilage volume was 4.7% with a 95% CI of 3.6% to 5.9% (133). Interestingly, initial cartilage volume was found to be a significant factor affecting cartilage loss and it was suggested that those in the early stages of OA lose cartilage more rapidly than those in the later stages of the disease (133). Similar results were found in a study by Raynauld et al. who evaluated cartilage loss over two years in 32 symptomatic osteoarthritic patients with similar demographics to the group studied by Wluka et al. in terms of age, weight, disease severity and clinical diagnosis (134). Over two years, a mean decrease of 7.6% (8.6%) was reported for the medial compartment of the knee, although it was not clear whether the "medial compartment" included the medial tibia or if it was just the loss in the medial femur. However, since it has been suggested that femoral and tibial cartilage volumes are highly correlated, it is likely that changes in femoral cartilage volume over time would be consistent with those of the tibia (135). Thus, the results would suggest a mean annual decrease in cartilage volume similar to that published by Wluka. In addition to these results, Raynauld et al. analyzed data further and found the presence of two distinct groups of individuals; a

group of 11 individuals labeled "slow progressors" and the remaining group of 21 individuals labeled "fast progressors". Those in the "slow" group lost very little medial cartilage over the two year period (<1%) while those in the "fast" group lost 17.9% (1.8%) of their initial cartilage volume over the study duration (134). As suggested by Wluka and others, these results support the notion that cartilage loss is not consistent in all individuals with knee OA (133).

In contrast to these results, Gandy et al. reported no significant change in cartilage volume in any knee compartment over three years in a group of 11 individuals suffering from knee OA (123). These individuals had predominant lateral compartment disease while one had patellofemoral OA at the end of the study. It is presumed that the remaining 8 individuals had medial compartment OA although this was not stated. There are a few possible reasons for the absence of observed decreases in cartilage volume over the study duration. As noted by the authors, this was a small sample size which may not be representative thus limiting the generalizability of the study. In addition, images from this study were acquired using a 1.0T MRI unit. While this group claims to have shown that data collected at 1.0T are comparable to those at 1.5T, the study of the comparison was conducted in healthy individuals and reported in abstract form only. Thus, one might question whether measurements are accurate and reproducible in those with knee OA. It is

also possible that, considering the small sample size, the population consisted of those who may, as suggested by Raynauld, be "slow" progressors, thus giving rise to negligible decreases in cartilage volume. Also, given the fact that these individuals had OA affecting different compartments of the knee, cartilage volume decreases in the medial compartment may have been overshadowed by those with decreases in the lateral compartment and no changes in the medial compartment. The presence of these study design issues lead one to question the accuracy of the results of this study and make the comparison of results between studies difficult. After considering the weaknesses of this study, it is likely that cartilage volume does, indeed, decrease with aging as suggested by the current study and supported by the results of Wluka and Raynauld (133;134).

Also in support of the results of this study is the study conducted by Cicuttini et al. (136). This study of 82 individuals with a spectrum of radiological OA severity as assessed by a standardized radiographic atlas, quantified medial tibial cartilage volume as it corresponded to grade of joint space narrowing. Results revealed that tibial cartilage volume consistently decreased with increasing joint space narrowing grade. For example, those with grade 0 had a mean (SD) tibial cartilage volume of 1.86 mL (0.86) while those with grade 1 had corresponding values of 1.52 mL (0.89 mL), grade 2 had values of 1.38 mL (0.65 mL) and those with

grade 3 had values of 0.74 mL (0.42 mL) (136). These results are consistent with those shown in Figure 38. Although corresponding analyses for cartilage thickness have never been reported, medial tibial cartilage thickness also consistently decreased with increasing K-L grade as shown in Figure 39.

It seems that increasing age and disease severity are associated with cartilage volume decreases and thinning cartilage but there is little consensus as to whether quantifiable cartilage parameters are associated with symptoms in those with knee OA. For instance, the relationship between cartilage volume and symptoms, as presented on the WOMAC questionnaire, was investigated in a group of 117 symptomatic individuals. At baseline, a weak inverse relationship was identified between pain and function and tibial cartilage volume at baseline with correlation coefficients of -0.17 ($p=0.05$) and -0.20 ($p=0.03$), respectively (137). Lindsey et al. also reported a significant negative correlation between medial tibial cartilage volume and thickness and WOMAC pain score ($r=-0.31$ for volume, $r=-0.33$ for thickness) in a group of 85 healthy and osteoarthritic individuals with varying degrees of OA severity (122). Results from these studies are consistent with those revealed in Table 18 where all variables of the WOMAC questionnaire and physical function on the SF-36 questionnaire were significantly correlated with medial tibial cartilage volume. However, the strength of these relationships was much greater in

the present study compared to those reported by Wluka et al. In a separate cross-sectional study conducted on 133 postmenopausal females from an unselected community-based sample population, only patellar cartilage volume significantly correlated with pain, function and global WOMAC scores whereas tibial and femoral cartilage volumes did not (138). However, it should be noted that females in this study were not necessarily osteoarthritic and only 97 of the participants experienced pain. Those without pain (pain scores = 0) were not included in the analyses. While cartilage volume was not quantified in a sample of 50 osteoarthritic patients also investigated for WOMAC scores, cartilage lesions were graded semiquantitatively and investigated for their relationship with symptoms. Results showed that the presence and severity of cartilage lesions were not significantly related to any WOMAC scores (139). From these three reports, only one study, in addition to the current one, quantified cartilage volume in an osteoarthritic population and related these symptoms to cartilage volume. In both cases, symptoms were found to correlate significantly with tibial cartilage volume.

Longitudinal data have also been reported correlating pain and symptoms with changes in cartilage volume over time. Wluka et al. showed very weak, yet significant, correlations between worsening of pain and functional capabilities and tibial cartilage loss ($r=-0.28$ and -0.21, respectively). In contrast, the study by Raynauld et al. observed no

significant correlation between changes in cartilage volume and worsening of symptoms as reported by the WOMAC scale and the physical function score on the SF-36 questionnaire, although subjects with more pain at baseline appeared to progress faster (134). Similarly, longitudinal cartilage loss (reflected by an increase in cartilage lesions) was not associated with changes in symptoms, as determined by the WOMAC score, in a group of 42 healthy and osteoarthritic individuals (140).

It is also important to note that the current study is the first to report the relationship between osteoarthritic symptoms and cartilage thickness in the knee joint. In fact, the correlations between pain, stiffness and functional ability as measured on the WOMAC scale, and physical function on the SF-36 with medial tibial cartilage thickness were all stronger than corresponding correlations with medial tibial cartilage volume. This may be due, at least in part, to the fact that volume is confounded by bone size whereas thickness is not. The strength of these relationships suggest that cartilage thickness may be just as important or even more important than volume to measure in osteoarthritic trials because of the strong correlation with symptoms. While this may be the case in cross-sectional studies as performed here, it is not known whether thickness measurements will correlate well with symptoms over time or whether cartilage thickness will change over time to the extent that volume may. Thus, further studies are needed to investigate the relationships between these variables over time

to determine whether cartilage thickness correlates more strongly with symptoms in longitudinal studies.

In comparing the strengths of the correlations between WOMAC outcomes and mJSW values and WOMAC outcomes and cartilage volume and thickness, it appears as though cartilage thickness is the most valuable quantitative outcome to accurately reflect symptoms of pain and function. Unfortunately, the association between mJSW and pain and stiffness was not consistently significant (as shown in Table 8) as compared to cartilage thickness which consistently showed a negative correlation with all WOMAC variables. In addition, the magnitude of the correlation coefficients appears slightly larger than those for cartilage thickness, although 95% CI overlap suggesting no significant difference. Based on these observations, it is suggested that cartilage thickness be used as an outcome measure in trials of disease-modifying osteoarthritic drugs because of its correlation with symptoms of pain and physical function.

While it is obvious from results such as these that MR imaging provides sufficiently detailed images of articular cartilage in order to quantify, using a software algorithm, the volume and thickness, it must also be recognized that MR images allow for the visualization and quantification of other bone and soft tissue abnormalities. The 3-D

gradient-echo imaging protocol applied in this study not only provided images of sufficient quality to measure medial tibial cartilage volume and thickness, but also the opportunity to identify other musculoskeletal features typical of OA including the presence of osteophytes and subchondral cysts and to assess the quality of the ligaments and menisci present in and around the joint. Although the fat-saturated gradient-echo MR images are less sensitive to marrow pathology than fat-saturated T2-weighted images due to magnetic susceptibility effects, they are sensitive in the assessment of menisci of the knee (141;142). In fact, results from multiple studies have revealed that gradient-echo imaging of the menisci has similar accuracy to spin-echo imaging techniques (143).

The prevalence of cartilage lesions in this asymptomatic population was relatively low (approximately 11%) compared to that reported by Zanetti et al. who revealed a prevalence of 25% in the asymptomatic knees of 100 individuals whose contralateral knee was suspected of having a meniscal tear (144). Perhaps the difference can be accounted for by differences in sample size and the notion that those with a suspected meniscal tear may be more likely to have a cartilage lesion in the contralateral knee than those who have two asymptomatic knees.

Also a characteristic feature of OA, osteophytes were found in 27% of MR images of asymptomatic individuals. Those identified as having

osteophytes on X-ray (K-L grade 2) also had evidence of osteophytes on MRI. In addition, 4 individuals with "possible osteophytic lipping" or a "doubtful osteophyte" on X-ray presented definite osteophytes on MR images. However, 8 other individuals who also had K-L grades of 1 did not show evidence of osteophytes on pMRI. The notion that 'doubtful' osteophytes are, indeed, "real" osteophytes was initially raised by Hart and Spector in their analysis of data from the Chingford Study (145). Results showed that 62% of women with baseline grade 1 K-L scores went on to develop true osteophytes ≤10 years after the initial X-ray. Using MRI, osteophytes were also identified in 5 individuals who showed no evidence of osteophytosis on X-ray. Because of the two dimensional nature of plain radiographs, it is likely that the true presence and prevalence of osteophytes identified on X-rays is underestimated (146). The lack of agreement between results from radiographs and MRI warrants further investigation.

Increased meniscal signal was the predominant abnormality in this asymptomatic group as evidenced by the fact that all but one individual showed evidence of a meniscal abnormality in at least one region of the knee. In fact, more than 60% of individuals exhibited an abnormality in at least 3 of the 4 regions. Data from previous studies reported meniscal abnormality prevalences of 16% to 67% (144;147-151). Consistent with the findings reported here, the posterior horn of the medial meniscus

appears to be the region most commonly identified with the largest number of degenerative changes (147;149;151). It is important to note, however, that the clinical significance of meniscal degeneration as defined here is not known. Although there were relatively few meniscal tears, it has been suggested that such tears are over-identified on MRI. Ninety-seven percent 97% of individuals in our study considered to be K-L grade 0 presented with a meniscal abnormality. Bhattacharyya et al. reported that 70% of those with medial meniscal tears had a K-L grade of 0 on X-rays. Our data suggest that the frequency and severity of abnormalities are increased in older individuals as compared to younger ones, as was also reported by Kornick and Fukuta (149;150).

Despite the small sample size and the fact that these seemingly asymptomatic volunteers were not clinically examined for a history of locking, swelling, varus or valgus deformity of the knee, these results suggest a high prevalence of meniscal degeneration in healthy individuals. Only recently has the importance of meniscal abnormalities been investigated with respect to the pathophysiology of knee OA (28;147). Thus, in addition to quantifying features of the knee such as cartilage volume and thickness, these results suggest it may be advantageous to identify those at risk for meniscal injuries. These results also suggest an underestimation of the prevalence of osteophytes identified on plain radiographs. However, plain radiography will likely remain the imaging

technique of choice for the clinical diagnosis and monitoring of disease progression due to the limited accessibility to MR scanners and their high operating costs.

The use of the three imaging modalities to evaluate various parameters of cartilage and bone have allowed reference ranges of mJSW, subchondral bone density and cartilage volume and thickness to be estimated and comparisons to be made between males and females and those with and without knee OA. To further our knowledge and investigate the potential for interactions between variables, further analyses were performed to identify the presence and strength of relationships between these variables. The first such relationship to be studied was that between medial tibiofemoral mJSW and BMD in the spine, hip, distal femur and proximal tibia.

Linear regression analyses were performed in healthy and osteoarthritic males and females to determine if, in fact, mJSW was significantly related to BMD. Results revealed that, in healthy females, mJSW was significantly positively related to BMD at the spine and hip with β-coefficients of 0.263 and 0.244, respectively. In healthy males, mJSW was positively related to hip BMD with a β-coefficient of 0.295. Relationships between these variables in healthy individuals have not been reported previously. Thus, it is interesting to note the presence of a

weak, yet positive significant relationship between BMD at the spine and hip and mJSW in the knee.

In osteoarthritic males and females, however, BMD at the spine and hip was not found to be significantly related to mJSW in the medial tibiofemoral compartment. This notion was also included in a report by Hannan et al. where BMD was not associated with joint space narrowing (152). While there have been very little data published on the relationships between these variables in healthy individuals, those with knee OA have been observed to have higher adjusted baseline BMD at the lumbar spine and proximal femur compared to those without OA (i.e. those with normal radiographs) (99;110;112-114). In addition, the Framingham and Chingford studies have shown that high BMD appears to be associated with incident knee OA while low BMD appears to be associated with OA progression (97;98;153). Due to the fact there were varying degrees of knee OA in the population studied for the thesis reflecting cases of newer or "incident" cases of OA as well as those who could be characterized as "progressors" (i.e. those who have had knee OA for a long period of time), it is quite possible that there was a mixture of higher and lower BMD values at the spine and hip leading to the absence of significant relationships. Thus, a re-analysis of the data was performed to investigate the relationship between K-L grades of knee OA, rather than mJSW, and BMD at the hip and spine. The appearance of these graphs,

as shown in Figures 47 and 48, indeed supports the results of previously published studies and affirms the fact that the presence of varying degrees of OA severity in the study population may have contributed to the initial lack of observation of significant relationships between mJSW and BMD were. The graphs suggest that those with K-L grade 2 OA, considered to have "early" or mild OA, have higher BMD values in both the spine and hip than those with K-L grade 3 and 4 OA, considered to be moderate and severe, although differences between K-L grades did not reach statistical significance ($p=0.10$).

Investigations of the relationships between BMD in the subchondral regions of the distal femur and proximal tibia with mJSW in the medial tibiofemoral compartment were also conducted. Here it was found that mJSW was significantly related to total distal femoral BMD as shown by a β-coefficient of 0.421 in healthy individuals. Likewise, mJSW was also found to be significantly related to proximal tibial BMD and the subchondral tibial region with β-coefficients of 0.408 and 0.311 in healthy individuals, respectively. These results suggest that those with higher BMD in the distal femur and proximal tibia have more space between the two opposing joint surfaces than those with lower BMD values. However, all BMD values lay within a "healthy range". It is possible that the relationship between BMD and mJSW in healthy individuals observed here may exist as a result of normal growth, meaning that those healthy

individuals with more "healthy" or "true" mineralization in the bone may also have thicker cartilage resulting in larger joint space widths, while those with less mineralized bone have thinning cartilage.

In comparison to healthy individuals, only BMD in the region of the subchondral femur (F4) was significantly associated with mJSW in those with knee OA, although here the associated β-coefficient was -0.426. Such a negative association means that those with high bone density in the subchondral femur have smaller mJSW values, reflecting more severe disease, compared to those with low bone density. This relationship, opposite to that observed in healthy individuals, has been observed in previously published studies where BMD around the affected joint is negatively correlated with joint space width in osteoarthritic patients (58;60;154). It has been proposed that accelerated bone turnover associated with knee OA results in thickening of the subchondral plate accompanied by an advancement of the tidemark in the subchondral region into the articular cartilage surface. Consequently, this advancement causes a further increase in the thickness of the mineralized subchondral plate and thus reduces the thickness of the articular cartilage (155). To reaffirm this observation, a graph depicting the relationship between K-L grade and BMD in the subchondral region of the distal femur is shown in Figure 27 and 28. Individuals with more severe disease (smaller mJSW) have higher BMD than those with early/mild knee OA.

A number of research groups have suggested reasons for the apparent presence of high bone density in the subchondral region surrounding an osteoarthritic joint (31;31;32;32;156). It has been suggested that subchondral bone appears to be denser in osteoarthritic individuals compared to healthy individuals. In fact the bone is less mineralized suggesting that the higher observed BMD is due to reasons other than increases in bone mineralization. This has recently been shown in a study of osteoarthritic hips by Mkukuma et al. where there was less mineral content in subchondral bone from an osteoarthritic hip than in healthy and osteoporotic individuals (157). Explanations for the apparent increases in bone density have been discussed in papers by Burr and Lajeunesse and Reboul (31;156). To summarize, bone density appears to increase because of an increase in subchondral trabecular bone volume and perhaps thicker trabeculae (158). It has been demonstrated that subchondral trabecular and cortical bone is less mineralized in those with OA compared to those without OA (159-161). In addition, collagen in subchondral bone is abnormal (156). Together with the higher ratio of α1 to α2 chains of type-1 collagen in the subchondral bone of osteoarthritic individuals as compared to non-osteoarthritic individuals, there is an over-hydroxylation of lysine residues in collagen fibrils. The decrease in cross-links observed in OA bone may help to explain the decrease in bone mineralization (156).

While the relationship between mJSW and BMD seems complex, the relationship between mJSW and articular cartilage volume and thickness is simpler since mJSW is considered a surrogate measure of cartilage thickness. Analyses were carried out to investigate the strength of the relationship between these parameters and to determine the proportion of mJSW variance that is accounted for by variance in medial tibial cartilage thickness. In both healthy and osteoarthritic participants, analyses showed that cartilage volume and thickness were significantly related to medial tibial mJSW as depicted in Tables 22 and 23. It is important to note that the two variables of concern are minimum JSW and mean cartilage volume and thickness. One might hypothesize that mean JSW would be more strongly correlated with mean volume and thickness or that minimum JSW might correlate more strongly with minimum cartilage thickness. Unfortunately, however, the cartilage segmentation algorithm does not quantify minimum cartilage thickness just as mean JSW was not calculated using the automated JSW algorithm. The fact that two different parameters are being correlated in these analyses must be taken into account and considered when interpreting these results.

Correlation analyses revealed two predominant observations, the first of which suggested that cartilage thickness is more strongly related to mJSW than cartilage volume in both healthy and osteoarthritic individuals.

This is possibly because of the fact that the confounding issue of bone size has been removed when focusing on cartilage thickness compared to volume. For instance, in considering healthy females, correlation coefficients were 0.55 for cartilage thickness compared to 0.51 for cartilage volume. In osteoarthritic females, the correlation between mJSW and cartilage thickness was 0.73 compared to 0.63. These trends were also apparent in males and in the entire population as a whole as shown in Tables 22 and 23. However, overlapping confidence intervals reveal that cartilage thickness is not, in fact, more strongly related to mJSW than is cartilage volume. Both variables were found to be strongly related to mJSW values in all groups who participated.

The second predominant observation was that correlation coefficients appeared slightly greater in osteoarthritic individuals than in healthy individuals, in healthy males compared to females and in osteoarthritic males compared to females. For instance, the coefficients reflecting the direction and strength of the association between mJSW and cartilage thickness was 0.80 in those with OA compared to 0.69 in healthy individuals as shown in Tables 22 and 23. Thus, approximately 64% of the variation in cartilage thickness could account for variation in mJSW in those with knee OA compared to only 48% in those who are healthy. Correlation coefficients also appeared stronger when comparing

correlation coefficients between healthy males and females and between osteoarthritic males and females.

After considering the values of the correlation coefficients and their associated confidence intervals, it is apparent that the confidence intervals overlap. Thus, cartilage thickness is no more strongly related to mJSW than is cartilage volume. Although correlation coefficients reflecting the relationships between medial tibia cartilage and thickness and mJSW were slightly larger in healthy males compared to females, in osteoarthritic males compared to osteoarthritic females and in osteoarthritic individuals compared to healthy ones, wide overlapping confidence intervals showed that this was not actually the case. In fact, power calculations were determined that the sample population was of inadequate size to determine if, in fact, significant differences between groups existed. Results showed that the sample was underpowered to be able to detect differences between healthy males and females (8%), osteoarthritic males and females (32%) and between osteoarthritic individuals and healthy individuals (30%).

To date, the majority of studies which have investigated the relationship between radiographic evidence of OA and evidence of OA as shown on MR have done so using semiquantitative scales of joint space narrowing, and cartilage volume and thickness. For instance, three

studies conducted by the Cicuttini group have shown that there is a strong, negative, linear association between medial tibial cartilage volume and increasing grade of joint space narrowing (136;162;163). In addition, a fourth study which analyzed both cartilage loss and joint space narrowing using semiquantitative scoring systems also evaluated the association between these variables over a period of 2 and a half years. Results showed that while the joint space narrowing score accurately reflected cartilage loss in the medial compartment with a specificity of 91%, its sensitivity was poor at only 23% suggesting that the joint space narrowing grade identified far fewer changes in cartilage loss than actually existed (164). Similar observations were also made in the study by Jones et al. (163).

To my knowledge, only two studies investigating the relationships between radiographic evidence of OA as reflected by mJSW measurements and cartilage volume and thickness have been published. For example, the study by Raynauld et al. (previously discussed) revealed a significant correlation of 0.47 between baseline mJSW in the medial tibiofemoral compartment and cartilage volume in the medial compartment (134). The strength of the association is similar to but slightly less than the correlation coefficient obtained in the current study between mJSW and medial tibial cartilage thickness in osteoarthritic individuals where r=0.61, although the confidence interval included the value of Raynauld et

al. (134). The other study which used JSW values in the correlation with cartilage thickness was conducted by Buckland-Wright et al. where cartilage thickness was highly correlated with joint space (165). In this study, however, it was noted that the joint space measurement was smaller than the cartilage thickness which suggested that the curvature of the femoral condyles compressed the cartilage upon standing. This is also of importance in the current study since X-rays were acquired in the weight-bearing fixed-flexion position and cartilage thickness measurements were made from non-weight-bearing MR images. It is possible that the tibial cartilage thickness was compressed during the X-ray as it was acquired in the weight-bearing position. Discrepancies in positioning during these two scans were not taken into account when investigating the relationships between these variables.

It must also be noted that medial tibial cartilage volume is not the only soft tissue which occupies the space between the distal femur and the proximal tibia. Since only 47% of the variation in mJSW in healthy individuals can be explained by variation in medial tibial cartilage thickness and only 64% can explain the variation in osteoarthritic individuals, there is a large amount of variation in mJSW which remained unexplained. Intuitively, one would hypothesize that medial femoral cartilage thickness would account for a portion of the remaining variation, and the study by Buckland-Wright supports this notion (165). However,

meniscal extrusion or subluxation has also been proposed as a factor which influences mJSW measurements and joint space narrowing (166-168).

Gale et al. studied the relationship between joint space narrowing and meniscal subluxation in a population of osteoarthritic and control subjects with a mean age of approximately 66 years (166). The results of this study showed that meniscal subluxation was significantly correlated with medial minimum joint space width as shown by a correlation coefficient of 0.55 (all subjects). The prevalence of meniscal subluxation was found to be very high (>80%) in cases and controls with identified joint space narrowing. Likewise, joint space narrowing was uncommon in those without meniscal subluxation (166). The results of the study by Adams et al. conducted in a group of 62 OA cases and controls were similar to those of Gale et al.; those showing evidence of joint space narrowing consistently had the highest grade of meniscal extrusion (167). A longitudinal study described previously showed that extrusion of the anterior horn of the meniscus was predictive of global and medial compartment cartilage volume loss in patients with knee OA (169). For example, in those with a severe medial anterior horn meniscal extrusion, the mean medial cartilage volume loss was 15.4 % compared to those without extrusion (4.5%) (169).

Results from these studies suggest that, in addition to cartilage thickness, meniscal subluxation/extrusion may play a significant role in determining mJSW measurements. Given the high prevalences of meniscal extrusions, it is likely that this variable also played an important role in the current study. Unfortunately, the MR protocol implemented in this study did not allow for assessment of this variable. Future studies would benefit from such measurements in helping to more clearly identify the proportion of mJSW accounted for by cartilage thickness in the tibia and femur.

While it is reasonable to assume that medial tibiofemoral mJSW is significantly positively correlated with medial tibial cartilage volume and thickness, the presence of a significant relationship between BMD in the spine, distal femur and proximal tibia and cartilage volume and thickness may not be as instinctive. However, investigating these relationships may help us understand whether the presence of knee OA affects bone systemically, in the axial skeleton, or whether bone surrounding the affected joint is the main site of altered activity. It is also fair to suggest that the relationship between BMD and cartilage volume and thickness might mimic the relationship between BMD and mJSW, since mJSW is a surrogate measure of cartilage thickness. After performing the correlation analyses, this was largely the case. In healthy females there was a significant, positive correlation between lumbar spine BMD and both

cartilage volume and thickness as seen by the β regression coefficients of 0.321 and 0.378, respectively. Although no such significant relationships were observed in males, when considered together, the relationship remained significantly positive. Only one other study has been conducted to examine the relationship between cartilage volume and BMD in a healthy population. In that study, Cicuttini et al. investigated the relationship between tibial cartilage volume and total body BMD in males and females without knee OA (mean age 55 years) (170). Tibial cartilage volume was found to be significantly associated with total body BMD in both males and females after adjusting for age, BMI and physical activity. In males, a similar positive correlation was found between cartilage volume and total hip BMD but not lumbar spine BMD (170). Unfortunately, the site-specific BMD measurements were not correlated with cartilage volume in females. Overall, however, the results are consistent with those of the thesis study.

Once again in females, mean distal femoral and proximal tibial BMD was found to be significantly related to medial tibial cartilage volume and thickness with β-coefficients of 0.292-0.323 for volume and 0.369-0.379 for thickness. In males, these relationships did not reach statistical significance. Overall, these results suggest that higher BMD in the spine as well as the mean distal femur and proximal tibia in healthy individuals are associated with greater cartilage volume and thickness in the medial

tibia. In addition, BMD in the subchondral regions of the distal femur and proximal tibia was not found to be significantly associated with cartilage volume or thickness in healthy individuals.

None of the relationships between lumbar spine, mean distal femoral and proximal tibial BMD and medial tibial cartilage volume and thickness were identified as being significant. However, BMD in the subchondral region of the femur was found to be significantly negatively associated with both medial tibial cartilage volume and thickness in males (β-coefficients of -0.713 for volume and -0.814 for thickness) as was the case with mJSW (β-coefficient = -0.582). In osteoarthritic females, however, no significant relationship was identified between subchondral femoral BMD and cartilage volume and thickness as was seen between subchondral femoral BMD and mJSW. Interestingly, BMD in the proximal tibial regions (i.e. mean or subchondral) was not significantly associated with cartilage volume and thickness or mJSW.

Generally speaking, results from this thesis suggest that bone surrounding the knee afflicted with OA is the primary region affected by the disease. It does not appear as though OA at the level of the joint affects bone metabolism in the spine. This is not surprising given the close proximity of the bone to the diseased joint. In fact, evidence from animal and human studies suggests subchondral bone located in closest

proximity to the joint may play a significant role in the initiation and/or progression of OA (29;30;103;154;156;158;171). The notion of irregular remodeling of subchondral bone in OA was described in a review by Lajeunesse and Reboul in which the evidence for abnormal osteoblastic metabolism was explored and the role of cytokines, growth factors and prostaglandins in the initiation and/or progression of OA was discussed (156). By leaking through fissures and channels in the bone to the bone-cartilage interface, these factors have the ability to stimulate cartilage breakdown, thus leading to decreases in cartilage volume and thickness (156;172;173). In addition, biochemical markers of bone formation and resorption have also been shown to be increased in patients with OA, possibly a reflection of abnormal remodeling and low bone mineralization (29;156;174-177). Although pre-clinical and clinical studies have shown that an increase in structural BMD is associated with OA, in actual fact it is suggested that the material density of the tissue is decreased (157). The apparent increase in BMD appears because the total volume of trabecular bone increases as a result of trabecular thickening, and the number of trabeculae may also increase reflected by the radiographic presence of subchondral sclerosis. However, what has been seen to actually occur is a decrease in mineralization of subchondral bone due to the increased rate of bone remodeling which does not allow the bone to fully mineralize, thereby reducing its stiffness (31;178).

The weaknesses associated with bone density acquisitions discussed previously also play a part in the inconsistencies in the results where, for example, significant relationships were identified in males, but not females. By improving the method of analyzing BMD in the distal and femur it is possible that results would be more accurate and reproducible.

What is evident from the discussion presented here is that significant associations exist between measures of bone and cartilage status in both healthy and osteoarthritic individuals, although the directions of these associations are not necessarily the same for these two groups of individuals. All three variables, mJSW, BMD and cartilage volume and thickness, allow important measures of disease status to be objectively evaluated and compared between different populations, such as between healthy and osteoarthritic individuals.

Chapter 8: Conclusions

The data and results from this study offer evidence that X-ray, DXA and MRI provide images of sufficient quality for the visualization and further quantification of mJSW, BMD and cartilage volume and thickness around the tibiofemoral joint in both healthy and osteoarthritic knees. Of these three techniques, X-ray measurements of minimum joint space were seemingly the quickest scans both to acquire and analyze, and proved to have the highest reproducibility of measurement. Bone density scans in and around the knee joint (subchondral bone) also proved to be quick and non-invasive, although determining BMD measurements in the distal femur and proximal tibia were time consuming (approximately 10 minutes/scan) and results were inconsistent likely because of variability in positioning during scan acquisition and the limitation of the analysis techniques. While MRI scans of the knee yielding measurements of cartilage volume and thickness in the medial compartment of the tibia may most accurately reflect the state of the cartilage in the knee due to its tomographic nature, segmentation analyses were also extremely time consuming and did not demonstrate impressive measures of inter-rater reliability. The nature of the manual segmentation was likely responsible for the both the time-consumption and the subjectivity of the determination of cartilage borders. Of all these techniques, perhaps the most encouraging future prospect is the automation of the cartilage

segmentation technique which will improve both the efficiency of measurement and, hopefully, also the inter-rater reliability. In so doing, it is possible that MRI could surpass plain radiography as the standard technique of diagnosis and monitoring disease progression. Unfortunately, however, access to MR equipment and their associated costs may be determining factors in the applicability of MR for clinical use.

The techniques available at the time this study commenced have offered valuable information to our knowledge of osteoarthritis and, perhaps just as important, have revealed promising areas of research requiring future investigation. Results of this study have both supported and negated hypotheses which were proposed in the introduction. In terms of mJSW, healthy males were indeed found to have larger values than females, both of whom have larger spaces than those afflicted with knee OA. These results were consistent with hypotheses. Comparisons between these populations suggest that there may be a threshold mJSW value below which individuals may be radiographically diagnosed with the disease. Once again, in males, this so called "cut-off" value is larger than in females. Contrary to the majority of previously published studies, mJSW values were also found to correlate with symptoms of pain and physical dysfunction, results which warrant further investigation. This observation is exciting because it lends support to the notion that there may be significant relationships between radiographic and symptomatic

measures of disease that have previously not been identified. Once again, however, future longitudinal studies are needed to confirm these observations.

Since measurements of mJSW in the tibiofemoral compartment are considered a surrogate measure of cartilage thickness, expectations that cartilage segmentation analyses would mimic or mirror mJSW values were found, for the most part, to be correct. Cartilage volume and thickness measurements were greater in males than in females and in healthy individuals compared to those with knee OA, just as was seen with mJSW measures. While cartilage volume and thickness values in healthy females appeared to decrease with age, this was not seen to be the case in males. However, in those with the disease, both volume and thickness decreased significantly with aging and increasing disease severity. Also similar to mJSW analyses were the findings that both cartilage volume and thickness correlated with OA symptoms of pain, stiffness and function as revealed by the WOMAC scores. In fact, this thesis is the first study to suggest that thickness measurements are more strongly correlated with arthritic symptoms than volume measurements. In addition, contrary to mJSW data, cartilage volume and thickness in males and females were significantly associated with knee stiffness. These results also lend support to the fact that there may be a stronger correlation between radiographic and symptomatic outcome measures than was once thought.

They also suggest that volume and thickness may be more sensitive measures of joint integrity than mJSW measures since correlations which were not statistically significantly associated with mJSW were significantly in relation to cartilage volume and thickness. However, whether these measures correlate over the long term and to what degree radiographic joint degeneration is reflected by symptoms of pain and function have yet to be determined and should be considered a future area of research. From these results, clinical trials of potentially disease modifying agents should consider studying both of these outcomes in order to determine changes in cartilage volume and thickness which may be considered clinically relevant.

Analyses undertaken to support or refute the hypothesis that mJSW measurements are significantly correlated with medial tibial cartilage volume and thickness indeed supported the claim. It is important to note, however, that cartilage thickness measurements reflected the mean cartilage thickness at the medial tibia whereas the joint space width measurement reflected the space where the cartilage was likely the thinnest. Thus, these two measurements do not necessarily reflect the exact same measurement and this would explain come of the variability in results. Despite this, results revealed that variance in medial tibial cartilage thickness accounts for only a proportion (53-77%) of the variance in mJSW measurement in those with knee OA and that femoral cartilage

and meniscal tissue likely account for the remainder. Between males and females these correlation coefficients were not identical as it appeared that a greater proportion of the variation in mJSW was accounted for by medial tibial cartilage thickness in males than in females both in healthy and osteoarthritic individuals. This may suggest that articular cartilage in females and males does not "wear" to the same degree in both sexes but that cartilage in the distal femur accounts for a greater percent of mJSW in one sex while cartilage in the proximal tibia accounts for a greater percent of mJSW in the other sex. This could be due to the gender differences in biomechanics or load bearing in the joint, although future studies would be needed to specifically study this hypothesis. In addition, further analyses to determine medial femoral cartilage volume and thickness would allow the determination of the proportion of minimum joint space accounted for by femoral cartilage are warranted. This might also help to determine the role of the menisci in joint space narrowing.

Bone mineral density results from this study were, for the most part, consistent with hypotheses. Although age and BMI were generally not found to be related to BMD in healthy males, they were found to be related in females. This is likely due to the small sample size of men in the study population as revealed by power calculations that were performed. While small differences between subchondral BMD values were found between those with and without knee OA, these differences were not statistically

significant. However, males with OA were found to have significantly higher BMD values in the distal femur and proximal tibia compared to osteoarthritic females. Data analyses also revealed that BMD in the subchondral regions of the distal femur and proximal tibia were also significantly related to disease severity (as evaluated on the K-L scale) as those with more severe disease had higher densities than those with milder disease. Inconsistencies in bone density results that were observed may be related to small sample sizes and inaccuracies in measurement techniques. However, results that were revealed suggest that further BMD analyses in the subchondral regions may be warranted with improved methodology.

In spite of the weaknesses in DXA methodology, analyses of the relationships between BMD and mJSW and cartilage volume and thickness were performed. Interestingly, in healthy individuals, BMD correlated positively with mJSW in the knee suggesting that those with higher areal density also have larger mJSW. For instance, of the subchondral regions, BMD in the proximal tibia was positively correlated with mJSW in the medial tibiofemoral compartment in healthy males and females. However, this relationship was reversed in those with knee OA where BMD correlated negatively with mJSW. Those with larger BMD measurements had increasingly narrowed mJSWs. This was the case in the distal femur where BMD was negatively related to mJSW, although

BMD in the proximal tibia was not. One possible explanation is that one goes through a phase of normal BMD with normal mJSW and cartilage volume. Then, in those who may be predisposed to developing knee OA, there is a loss of bone reflected in a decline in BMD, which later reverses itself when the sclerotic phase of OA sets in. If this hypothesis is actually the case, then the optimal time to intervene in the disease process may be when the BMD begins to fall. Once again, improved methodology and increases in sample size may help to clarify these relationships and may provide more information as to the justification or explanation behind the observations.

Significant positive relationships between cartilage volume and thickness and subchondral BMD were also identified. In fact, both the total distal femoral BMD and the total proximal tibial BMD were significantly related to medial tibial cartilage volume and thickness in healthy individuals. Once again, just as seen in mJSW analyses, subchondral BMD in the distal femur and proximal tibia was negatively related to cartilage volume and thickness values of the medial tibia, results which support observations seen between mJSW and BMD analyses in those with knee OA. It is hopeful that these clinical results will warrant further basic science experimentation to try to explain the pathophysiology behind these observations.

While a few of the methodological limitations of this study have already been discussed, it is important to explain that these results were, for the most part, yielded from a sample of individuals who may not be totally representative of either a healthy or osteoarthritic community population. All healthy participants were those who volunteered to participate in the study and represented, for the most part, a healthy, educated, middle-class population with very few comorbidities (i.e. smoking status). Thus, these individuals may represent a slightly biased sample, one which is generally healthier compared than what would be considered a cross-section of community dwelling individuals. A cross-section population would likely include a wider variation in socio-economic status, health status, etc. Likewise, the vast majority of individuals with knee OA who participated were being treated by rheumatologists and, thus, were not representative of a cross-section of those with OA from the community. The relatively small number of osteoarthritic individuals who participated in the study, especially those under 50 years and over 70 years, may have limited the power of the analyses to detect differences where they may actually exist. Thus, future studies should include larger sample sizes of these individuals. In so doing this would also allow for the analyses of more potential covariates (i.e. alcohol intake and exercise status) while maintaining an adequate power.

In spite of these limitations, these important results are revealed at a time where the arrival of disease modifying osteoarthritic agents is imminent and there is a significant need for reference values/ranges of radiographic data. Not only will outcome measures of mJSW, BMD and cartilage volume and thickness be used in inclusion and exclusion criteria in clinical studies, but they will also be used to support or refute the notion that pharmacological agents indeed have disease modifying properties. With the number of people affected by this debilitating disease increasing and life expectancy on the increase, the demand for agents with both disease and symptoms modifying properties will increase in parallel. It is only with advances in imaging and analysis techniques that these agents will be able to be marketed as remedies which treat the patient and the disease simultaneously.

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Figures & Tables

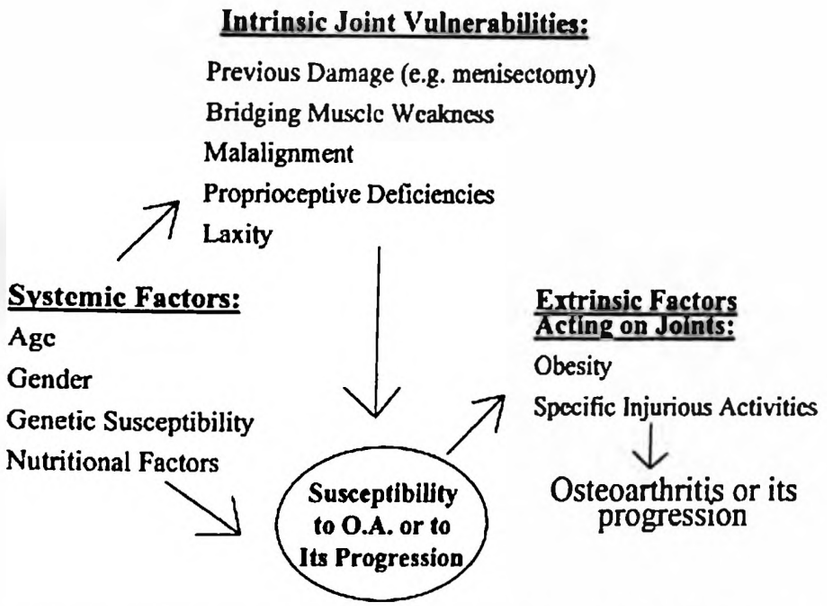


Figure 1: A model of the role of intrinsic and extrinsic factors in the pathophysiology of OA proposed by Felson (1)

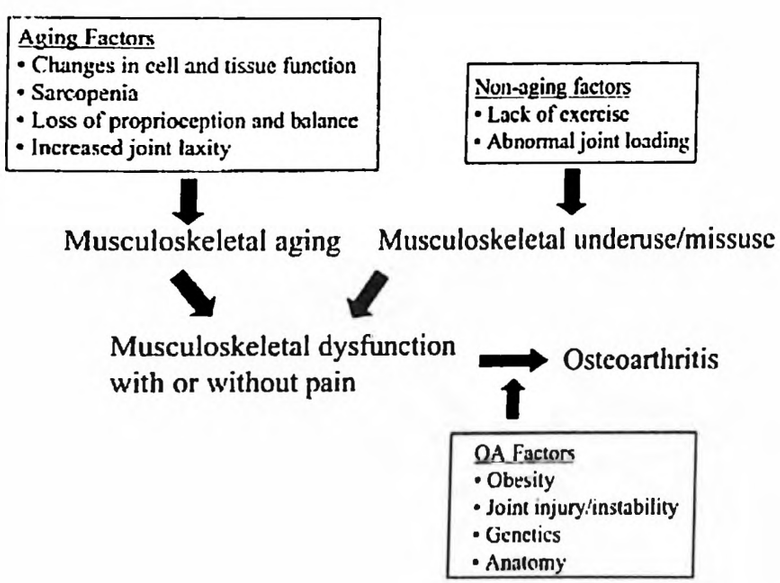


Figure 2: A model of the role of intrinsic and extrinsic factors in the pathophysiology of OA proposed by Loeser (5)

Table 1: Kellgren-Lawrence scale for grading of plain radiographs

K-L Grade	Definition
0	absence of osteophytes and normal joint space width
1	doubtful narrowing of joint space and possible osteophytic lipping
2	definite osteophytes and possible narrowing of joint space
3	moderate multiple osteophytes, definite narrowing of joints space, some sclerosis and possible deformity of bone contour
4	large osteophytes, marked narrowing of joint space, severe sclerosis and definite deformity of bone contour

Table 2: Comparison of various radiographic positioning techniques

Position	Fluoro use?	Knee Flexion	Angle of X-ray	Foot Angle	Distance to Table
Semi-flexed (B)	Yes	10-20° (tib. plateau ‖ to floor & X-ray beam)	‖ to floor	medial tibial plateau ‖ to X-ray & tibial spines under femoral notch (≈15° externally)	unknown
Lyon-Schuss (C)	Yes	20-30° (optimize med. tib. plateau alignment)	‖ to tibial plateau		Great toes, patellas & thighs coplanar with & touching table
MTP (A)	No	7-10°	Parallel to floor	15° externally	MTP & patellas touching table
Fixed Flexion	No	Fixed by femur, tibia & great toe positioning (≈20°)	‖ to tib. plateau (≈10° caudal)	10° externally	Great toes, patellas & thighs touching table

‖ means parallel, ≈ means approximately

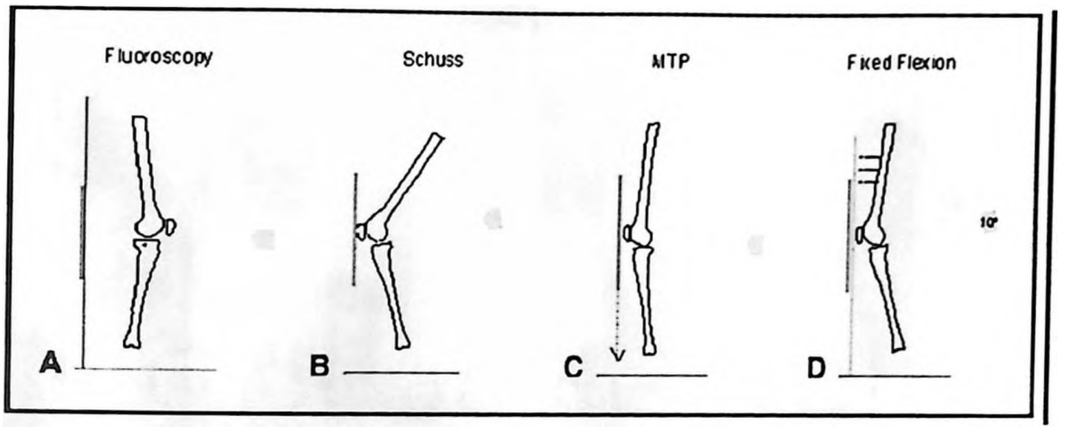


Figure 3: Radiographic positioning techniques. A: Semiflexed, B: Lyon Schuss, C: MTP and D: Fixed Flexion X-ray positioning techniques. Note in the Fixed Flexion technique, that the great toes, knees and thighs are tightly pressed against the vertical table fixing the angulation of the tibia and femur (28).

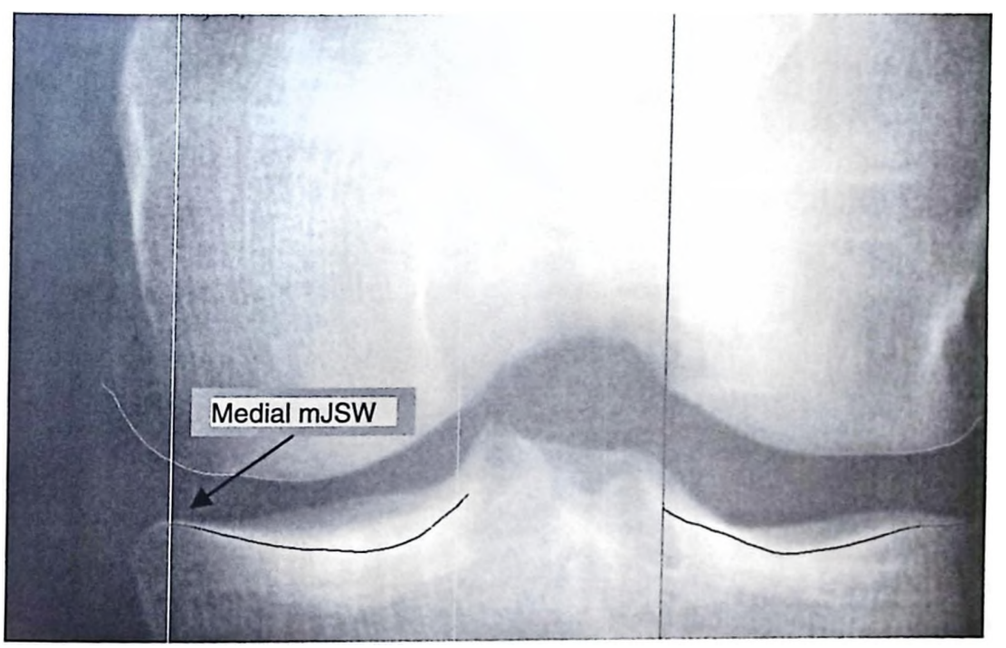


Figure 4: Analysis of a digitized radiograph using the automated software algorithm. The algorithm delineates the medial (left) and lateral (right) compartments of the knee, the femoral condyles and the tibial plateau. The red lines indicated the mJSW in the medial and lateral compartments.

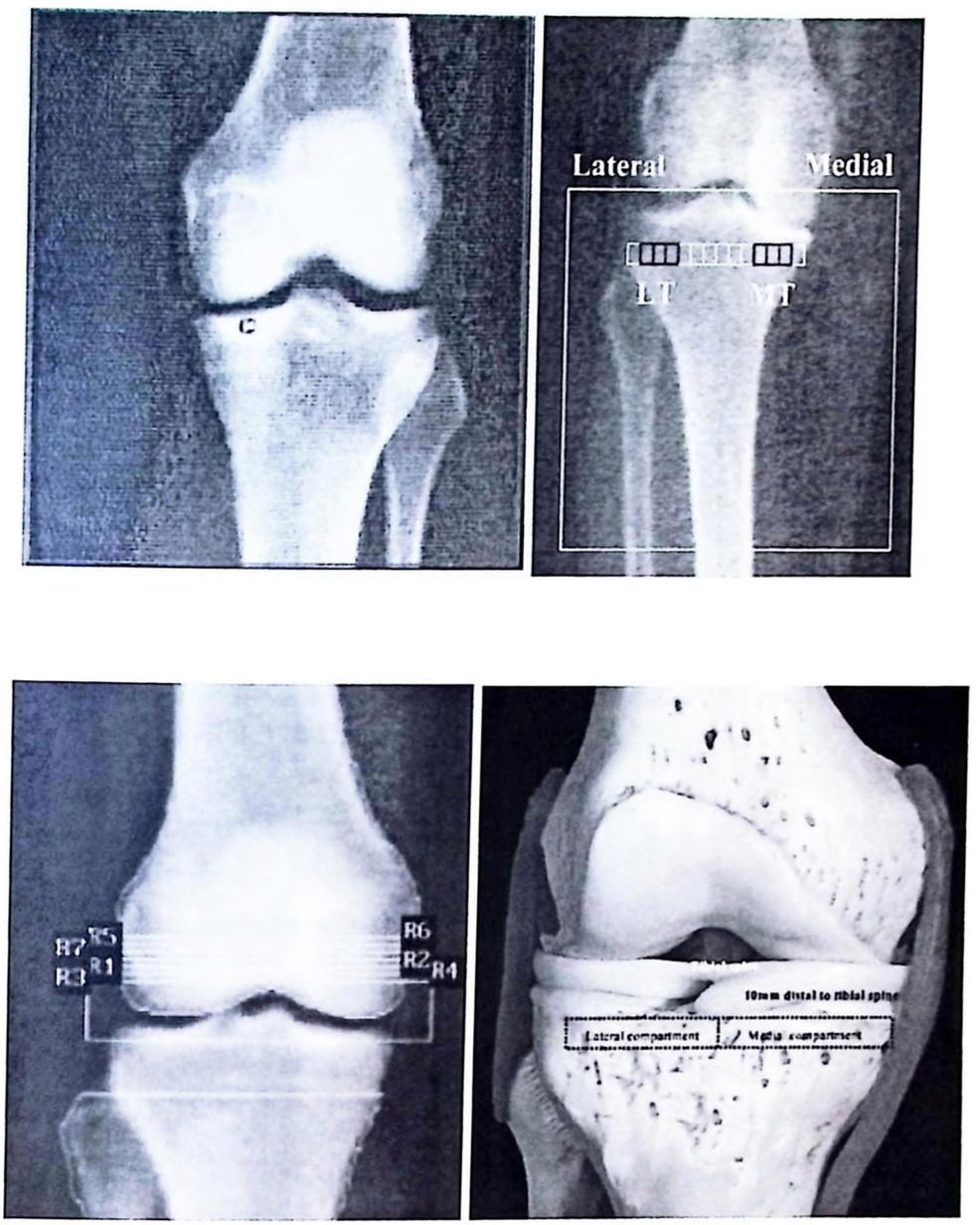


Figure 5: Previously published techniques for quantifying BMD in areas of the subchondral tibia. Note the variably selected regions of interest of fixed sizes and heights (48;58-60). It is evident that the size, number and location of the regions of interest differ in each study.

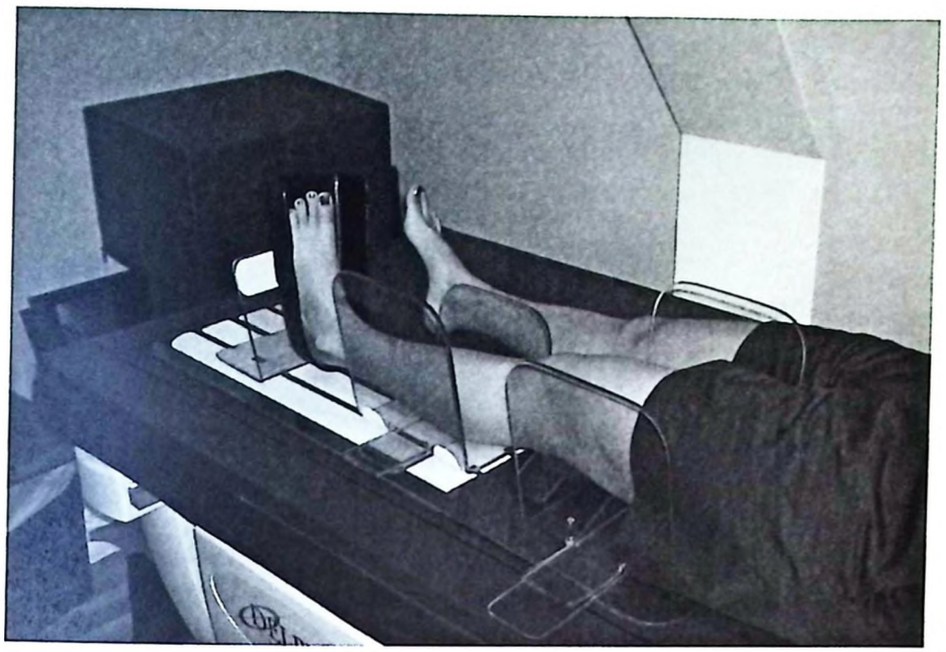


Figure 6: The polycarbonate positioning device used to position the thigh and leg for DXA scanning

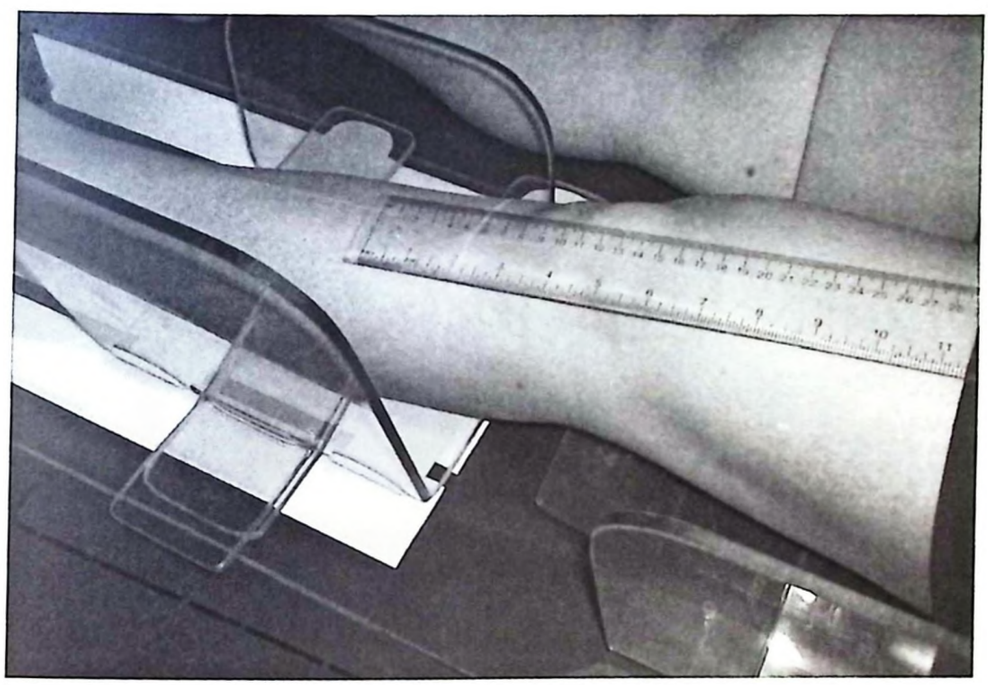


Figure 7: Placement of the laser crosshair on the proximal tibia for scanning of the proximal femur

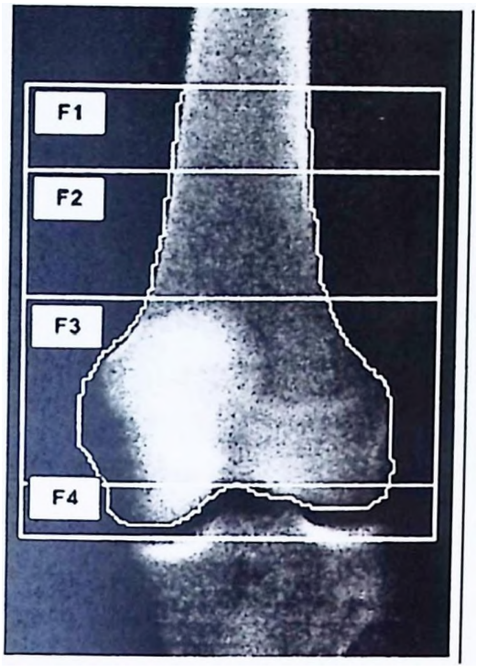


Figure 8: Analysis of bone mineral density in the distal femur

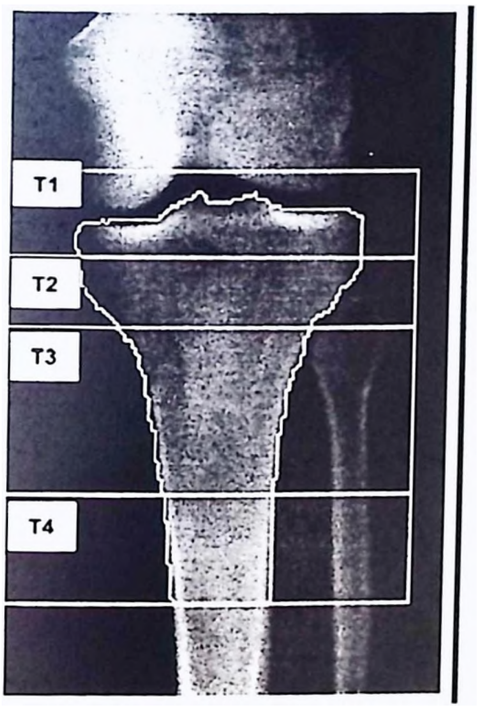


Figure 9: Analysis of bone mineral density in the proximal tibia

Figure 10: OrthOne™ peripheral MRI scanner used to image knees of study participants

Table 3: Accuracy of segmentation algorithms for quantification of articular cartilage volume and thickness

	Resolution (mm)	Parameter	Region of Knee	Pairwise Random Difference
Cicuttini (130)	0.3 x 0.3	Volume	Total Femur	9.2%
			Total Tibia	9.2%
Cicuttini (64)	0.3 x 0.6	Volume	Total Femur	2.0%
			Total Tibia	3.2%
Eckstein (179)	0.3 x 0.3	Thickness	Medial Tibia	17.9%
			Lateral Tibia	13.7%
Marshall (180)	0.3 x 0.8	Thickness	Medial Femur	3.6%

Table 4: Reproducibility of segmentation algorithms for quantification of articular cartilage volume

	Resolution (mm)	Subjects (N)	Region of Knee	Coefficient of Variation
Cicuttini (64)	0.3 x 0.6	Healthy (10)	Total Femur	2.6%
			Total Tibia	2.6%
		Osteoarthritis (8)	Total Femur	2.9%
			Total Tibia	3.2%
Eckstein (181)	0.3 x 0.3	Healthy (14)	Total Femur	2.3%
			Medial Femur	4.9%
			Lateral Femur	5.3%
			Medial Tibia	2.5%
			Lateral Tibia	2.3%

$$\textbf{RMSSD} = \text{Square Root } \frac{\sum (x1_j - x2_j)^2}{2N} \qquad \textbf{CV} = \frac{\text{RMSSD}}{\frac{\sum (x1_j + x2_j)}{2N}} \times 100\%$$

Figure 11: Methods for calculating RMSSD and CV; $x1_i$ and $x2_i$ are the two measurements for knee *i (i=1 up to N)*, and *N* is the total number of duplicate measurements. (47)

Table 5: Reproducibility of mJSW by computer and independent readers

	Computer mJSW		Reader 1 mJSW		Reader 2 mJSW	
	RMSSD (mm)	CV (%)	RMSSD (mm)	CV (%)	RMSSD (mm)	CV (%)
Short-Term						
ALL	0.19	4.41	0.20	4.99	0.25	5.69
Healthy (KL 0 or 1)	0.15	3.31	0.15	3.31	0.19	4.12
OA (KL 2 or 3)	0.27	7.45	0.29	8.10	0.35	9.95
Long-Term	0.14	2.86	0.17	3.35	0.17	3.34

RMSSD = root-mean-square standard deviation, CV = coefficient of variation

Table 6: mJSW data per sex and decade of life in healthy individuals

	Age Group (yrs)	N	Mean (mm)	SD (mm)	Range (mm)	Min. (mm)	Max. (mm)
Females	20 - 29	22	5.06	.56	1.92	4.34	6.26
	30 - 39	15	4.62	.656	2.16	3.49	5.65
	40 - 49	14	4.84	.69	2.71	3.81	6.52
	50 - 59	17	4.75	.93	3.77	3.57	7.34
	60 - 69	5	4.61	.44	1.13	3.99	5.11
Males	20 - 29	18	5.55	.51	1.97	4.57	6.53
	30 - 39	12	5.76	.71	1.94	4.70	6.64
	40 - 49	8	5.35	1.08	3.60	3.57	7.16
	50 - 59	6	5.43	.71	2.05	4.35	6.40
	60 - 69	2	5.41	.56	1.88	4.47	6.35

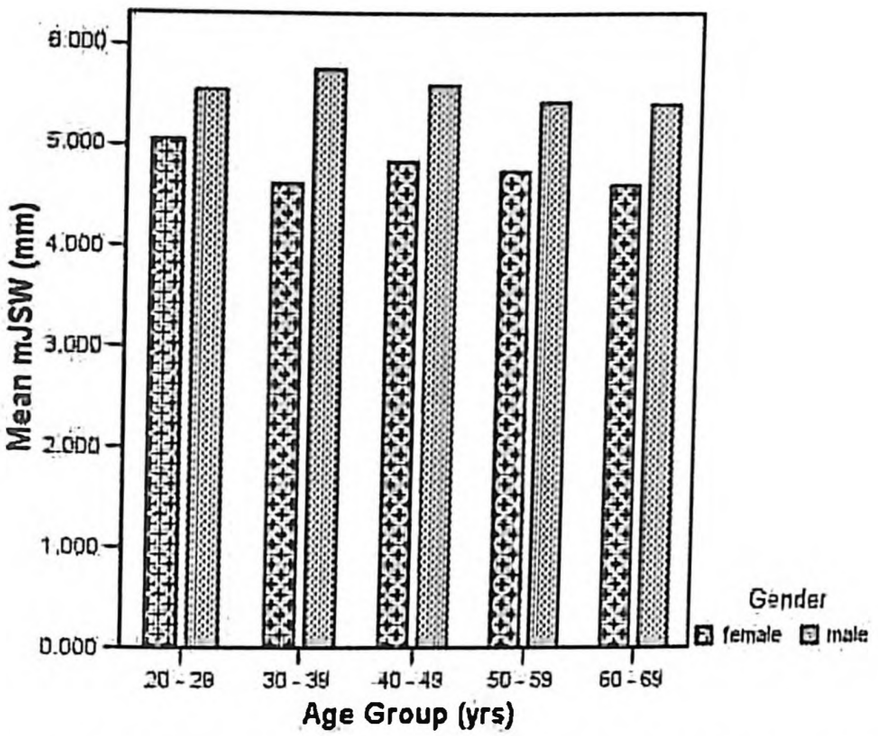


Figure 12: Average mJSW values in each decade of life in healthy females and males

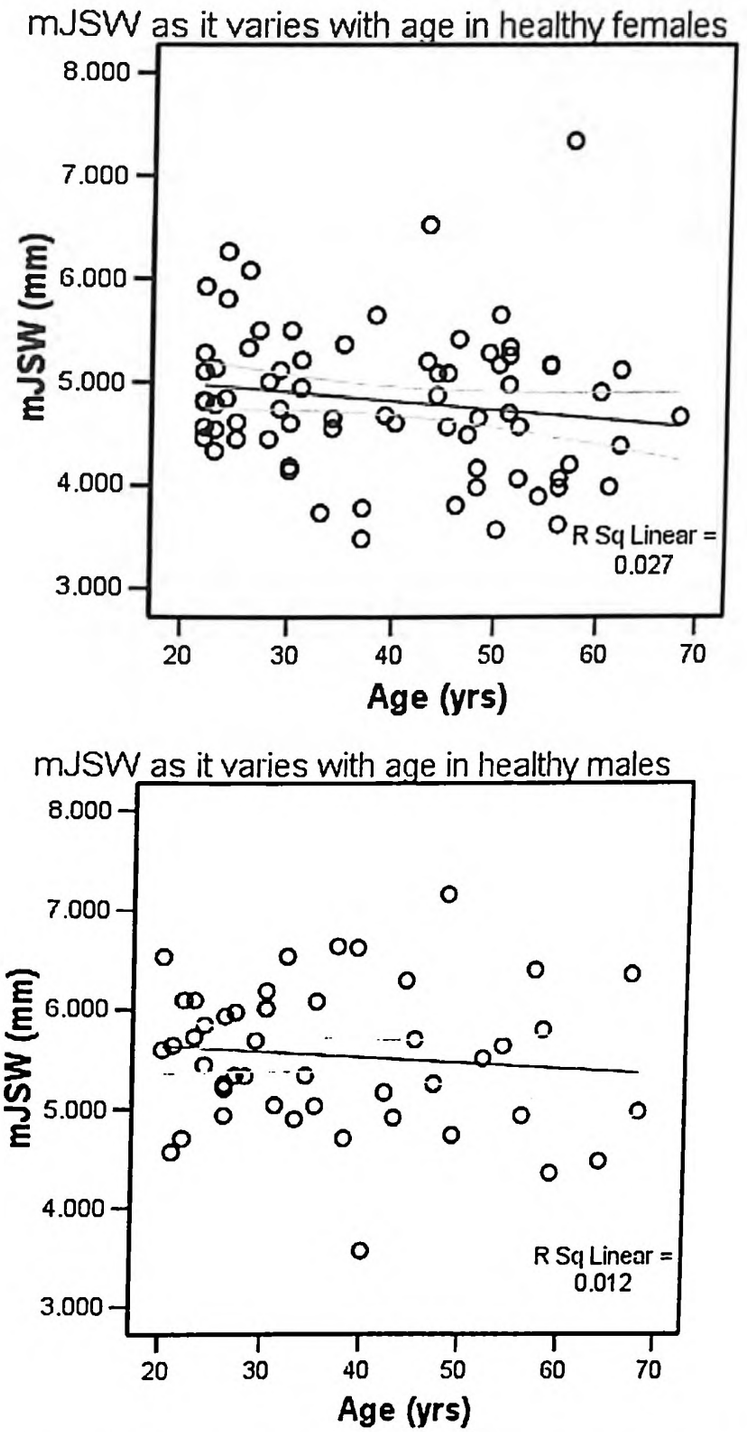


Figure 13: Age distribution of mJSW in healthy females and males. Two outlier points, indicated by surrounding boxes, are depicted in the graph of healthy females.

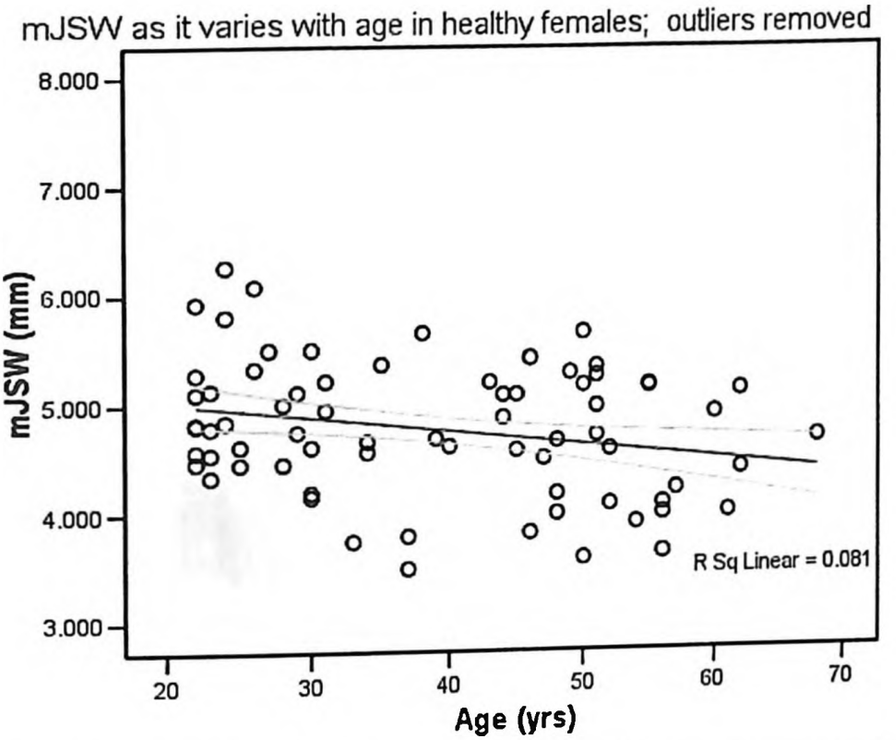


Figure 14: Age distribution of mJSW in healthy female group after removal of outlier

Table 7: mJSW data per sex and decade of life in osteoarthritic individuals

	Age Group (yrs)	N	Mean (mm)	SD (mm)	Range (mm)	Min. (mm)	Max. (mm)
Females	30 - 39	1	5.75	n/a	.000	5.75	5.75
	40 - 49	3	4.54	0.27	0.51	4.23	4.74
	50 - 59	13	4.17	1.29	4.49	1.87	6.35
	60 - 69	9	3.30	1.77	6.01	.00	6.01
	70 - 79	5	1.85	1.83	3.98	.00	3.98
	80 - 89	2	0.64	0.90	1.27	.00	1.27
Males	40 - 49	2	4.45	1.34	1.89	3.51	5.40
	50 - 59	5	4.05	2.74	6.44	.00	6.44
	60 - 69	8	5.10	0.99	3.39	4.00	7.39
	70 - 79	7	1.33	1.92	4.15	.00	4.15

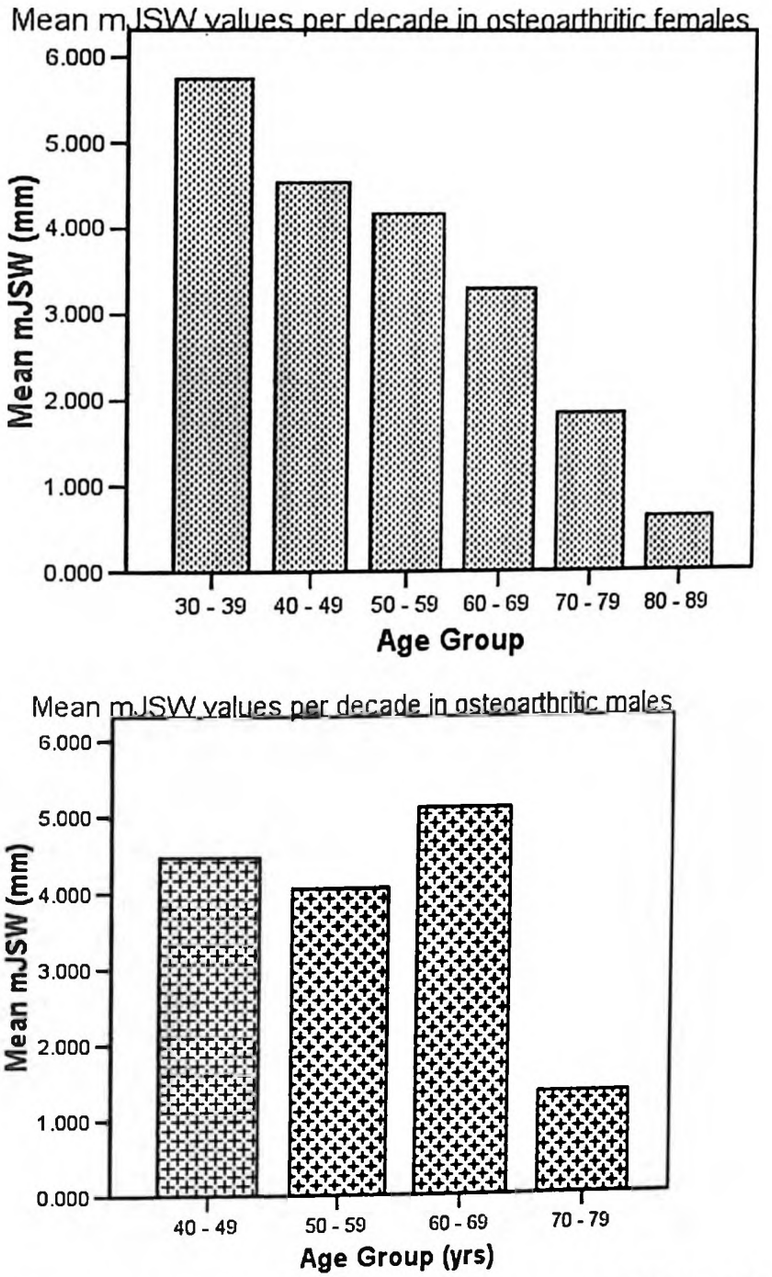


Figure 15: Average mJSW in each decade of life in osteoarthritic females and males

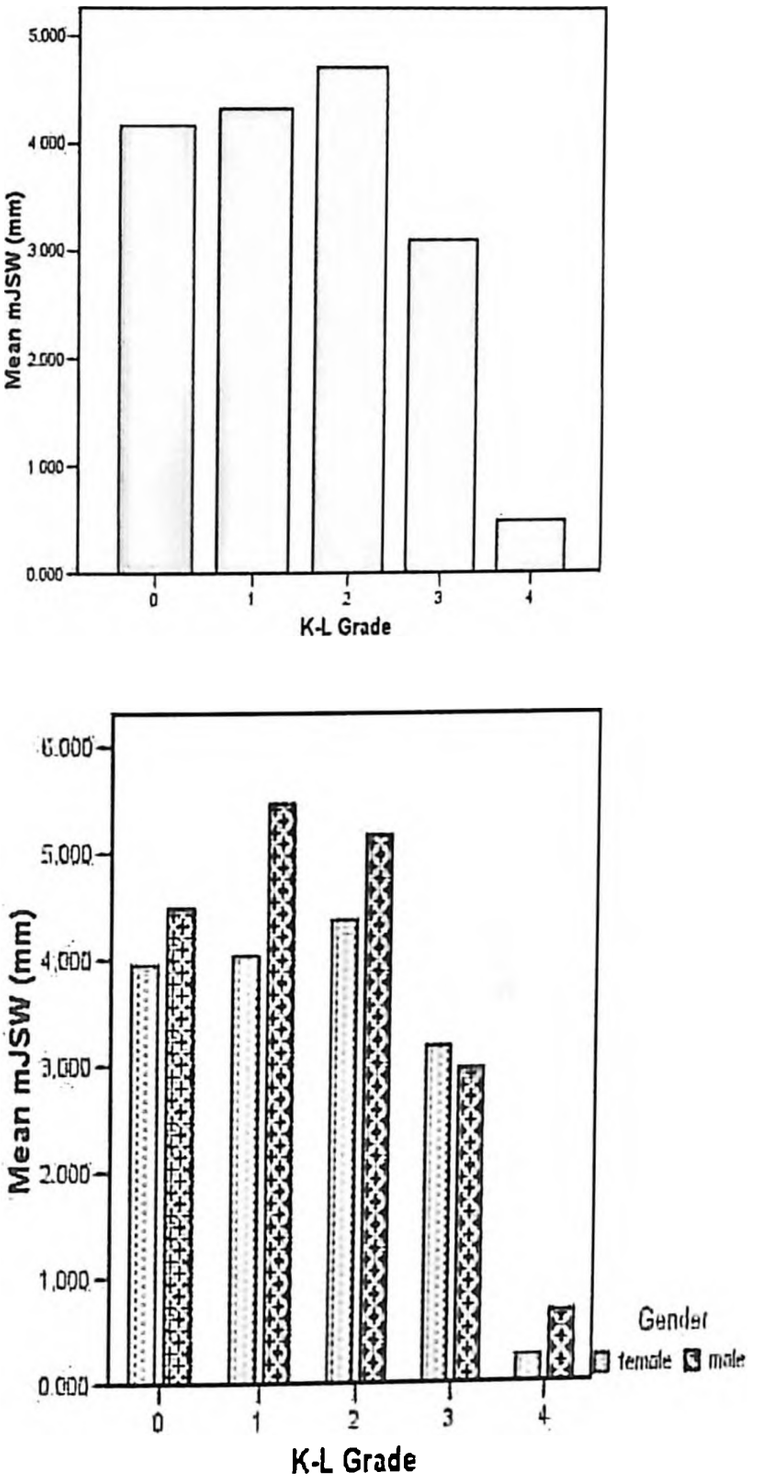


Figure 16: The distribution of mJSW as it relates to K-L grade in osteoarthritic individuals

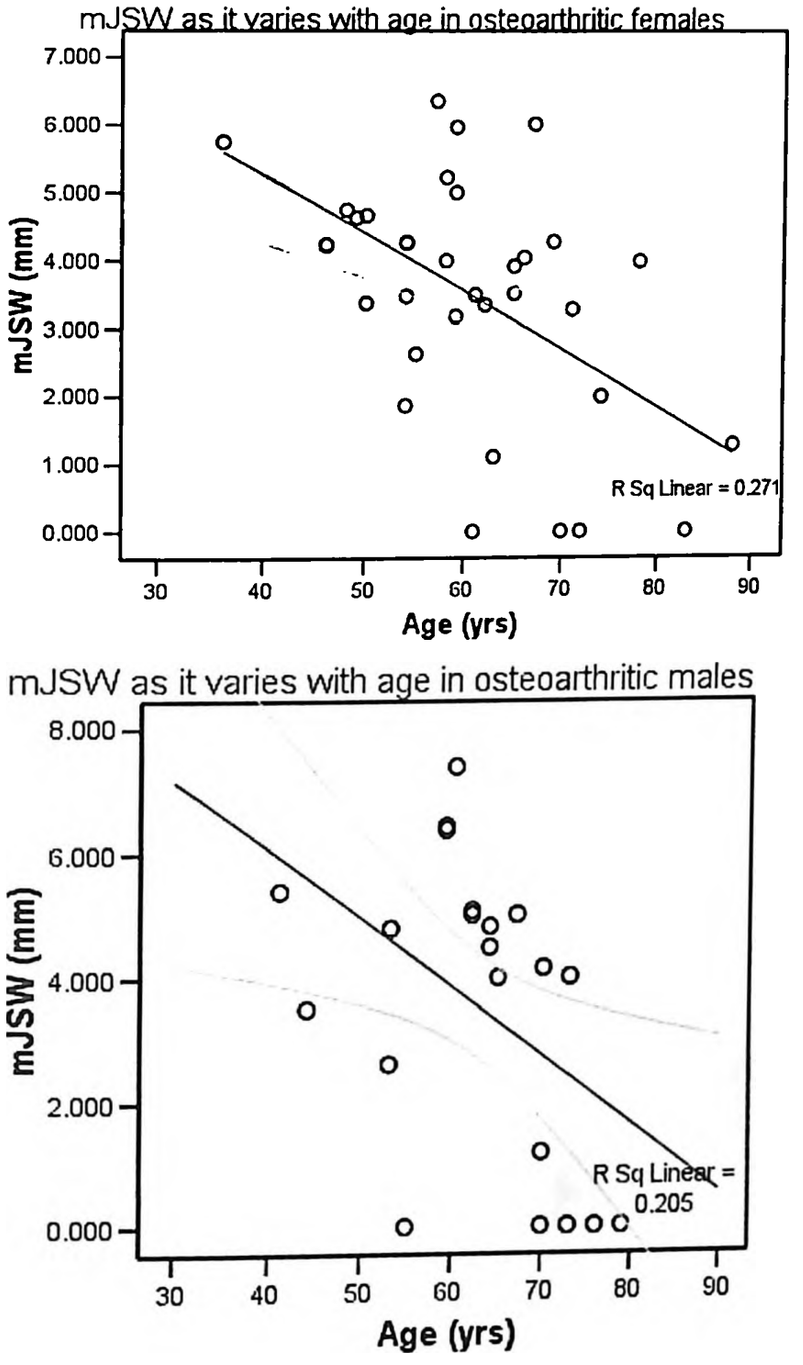


Figure 17: Age distribution of mJSW in osteoarthritic females and males

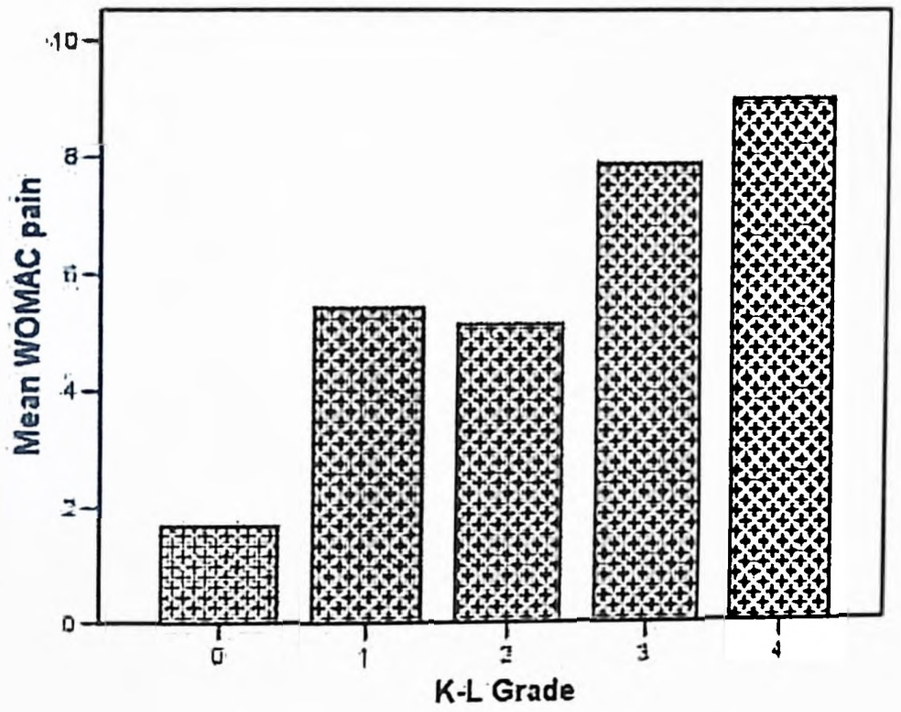


Figure 18: Relationship between K-L grade and WOMAC pain scores in those with OA

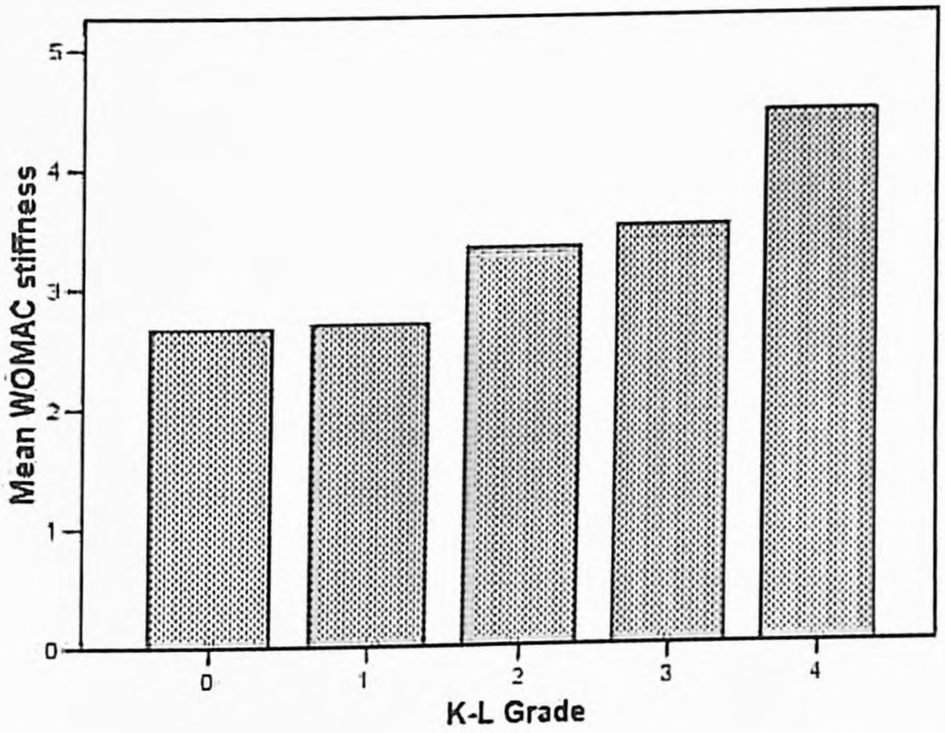


Figure 19: Relationship between K-L grade and WOMAC stiffness scores in those with OA

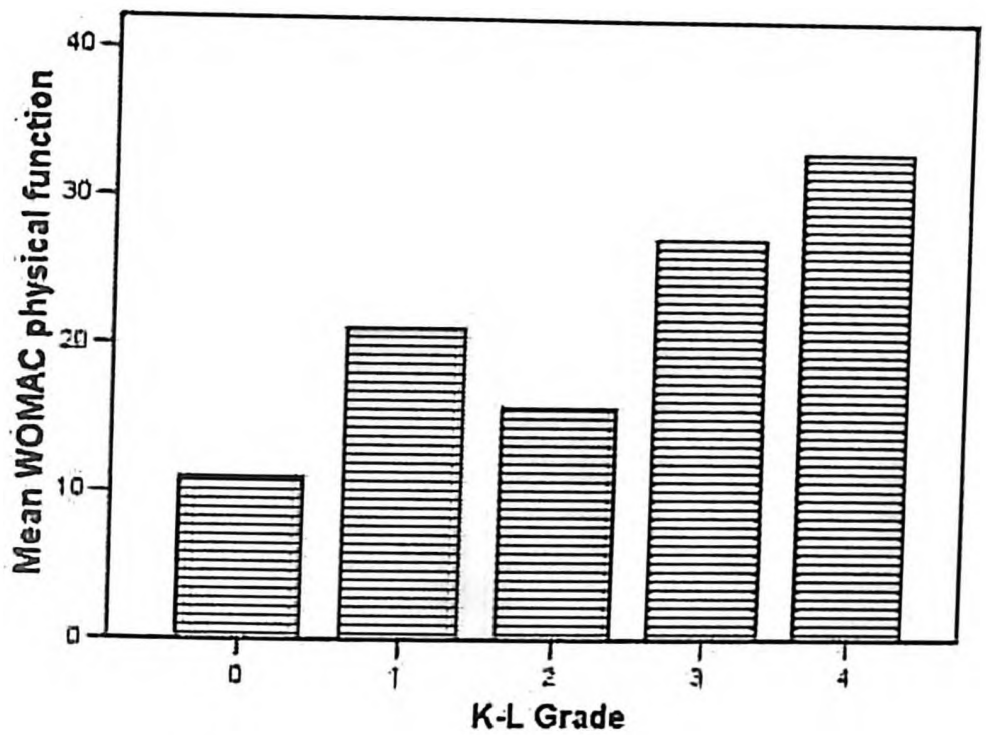


Figure 20: Relationship between K-L grade and WOMAC physical function scores in those with OA

Table 8: Relationships between mJSW and pain, stiffness and function (WOMAC questionnaire)

	Females		Males	
	CC (r)	**95% CI of r**	**CC (r)**	**95% CI of r**
Pain	-0.500	-0.743, -0.14	-0.474*	N/A
Stiffness	-0.377*	N/A	-0.411*	N/A
Function Ability	-0.530	-0.761, -0.180	-0.705	-0.894, -0.302
Total WOMAC	-0.528	-0.759, -0.177	-0.662	-0.876, -0.227

CC = Correlation Coefficient, * = not statistically significant ($p<0.05$)

Table 9: Bone mineral density values in healthy individuals

	Region	N	Mean (g/cm^2)	SD (g/cm^2)	Range (g/cm^2)	Min. (g/cm^2)	Max. (g/cm^2)
Females	L-Spine	57	1.040	.098	.411	.800	1.211
	Total Hip	57	0.969	.124	.776	.557	1.333
Males	L-Spine	37	1.071	.157	.543	.841	1.384
	Total Hip	37	1.082	.175	.696	.775	1.471

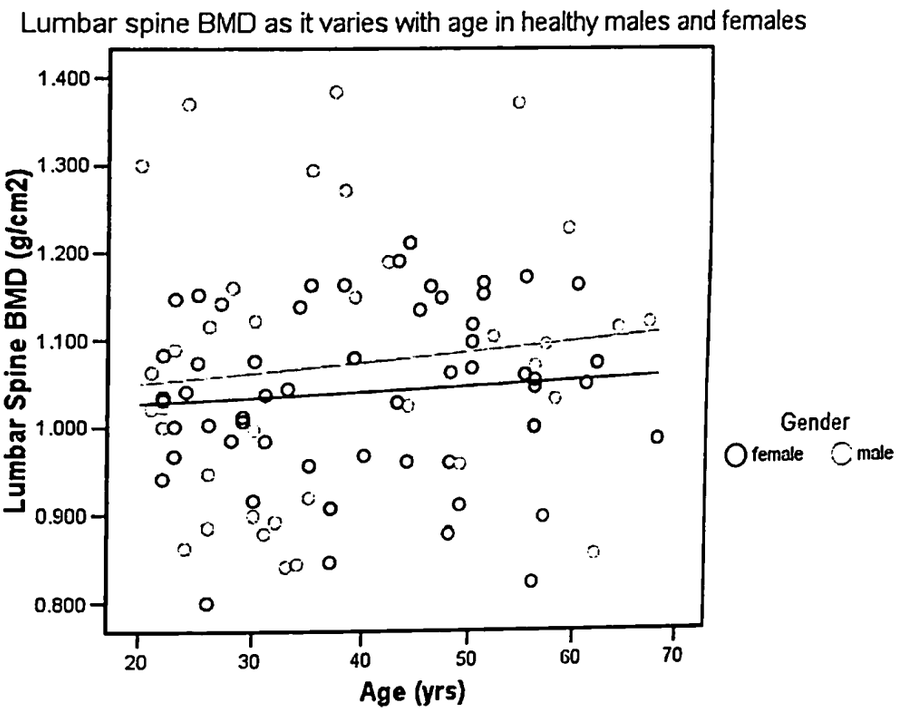


Figure 21: Relationship between L-spine BMD and age in healthy volunteers

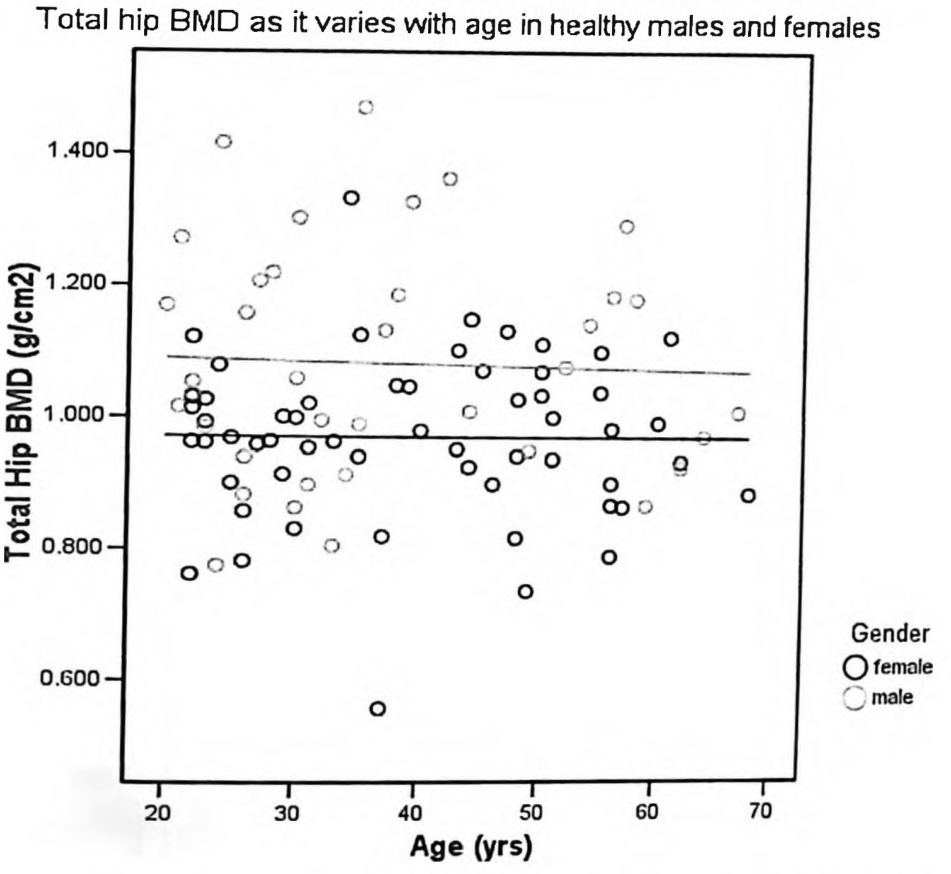


Figure 22: Relationship between total hip BMD and age in healthy volunteers

Table 10: Bone mineral density values in healthy individuals

	Region	N	Mean (g/cm^2)	SD (g/cm^2)	Range (g/cm^2)	Min. (g/cm^2)	Max. (g/cm^2)
Females	F- F4	55	0.939	.107	.514	.617	1.131
	F- Total	55	1.007	.112	.508	.765	1.273
	T- T1	54	0.897	.113	.538	.608	1.146
	T- Total	54	0.943	.110	.493	.657	1.150
Males	F- F4	35	0.938	.095	.395	.730	1.125
	F-Total	35	1.119	.124	.501	.874	1.375
	T-T1	34	0.958	.161	.718	.536	1.254
	T-Total	34	1.044	.178	.700	.730	1.430

F = Femur, T = Tibia

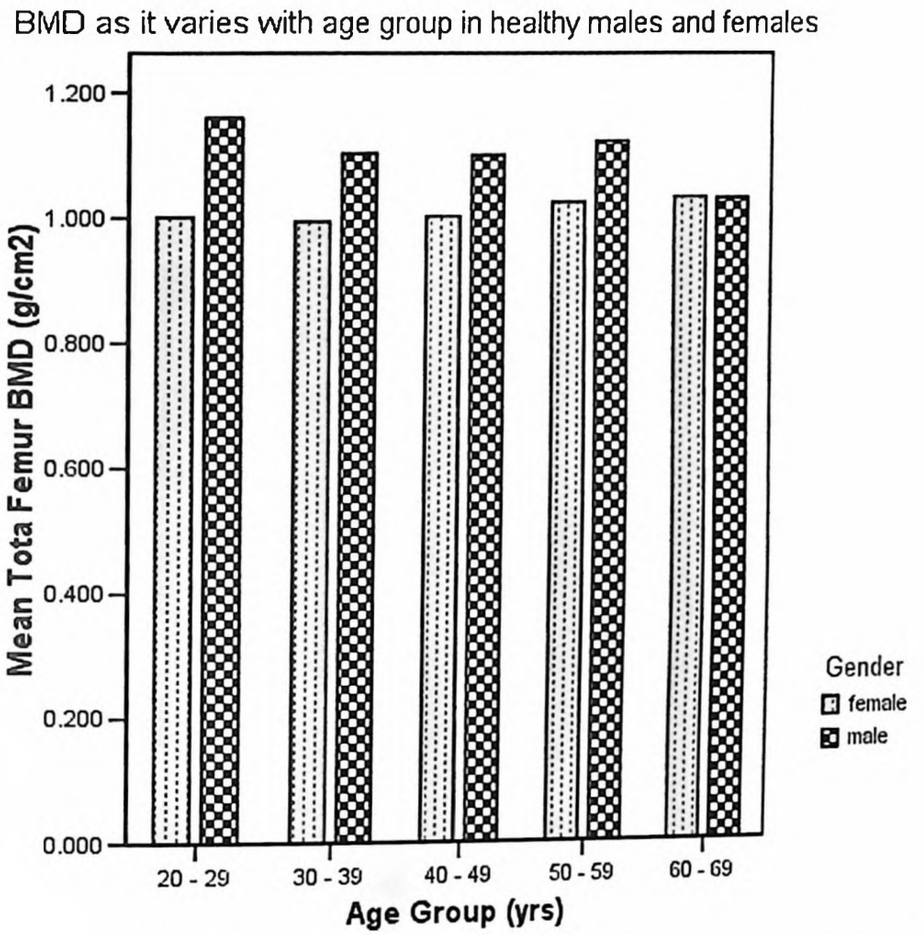


Figure 23: Mean distal femoral BMD per decade in healthy males & females

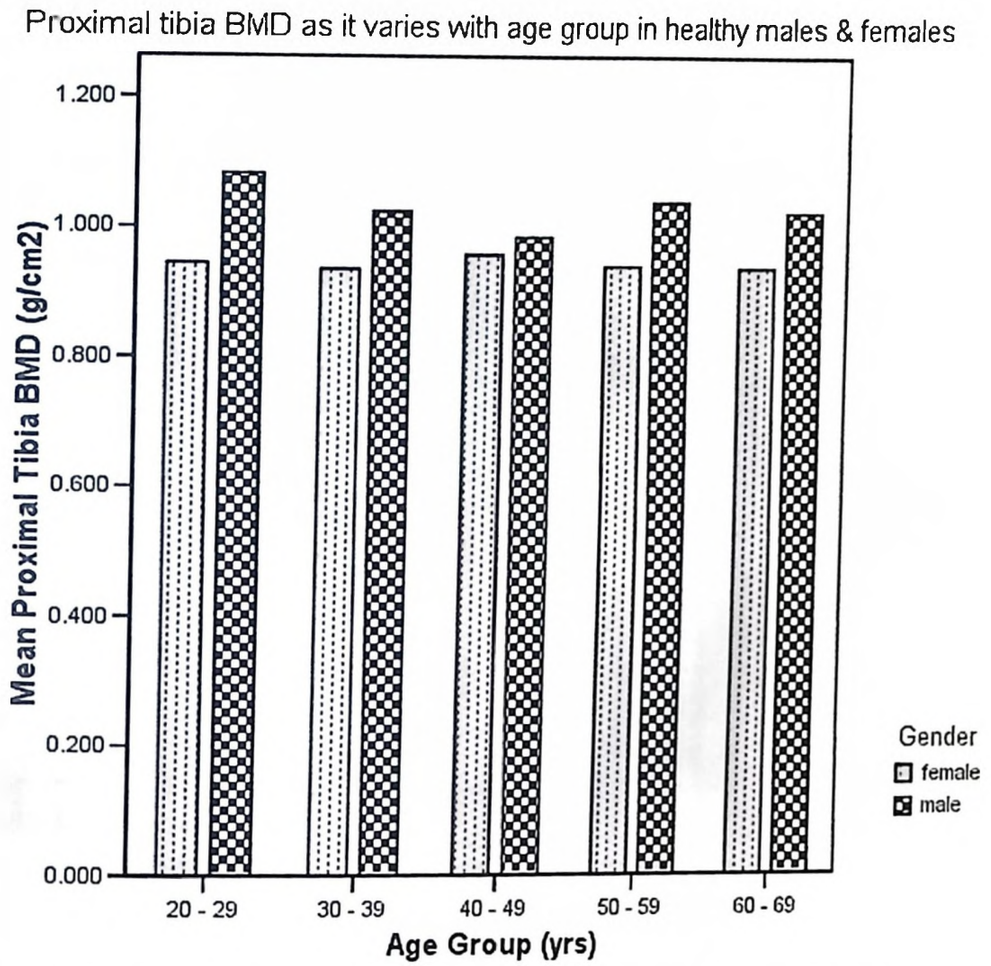


Figure 24: Mean proximal tibial BMD per decade in healthy males & females

Table 11: Bone mineral density values in osteoarthritic individuals

	Region	N	Mean (g/cm^2)	SD (g/cm^2)	Range (g/cm^2)	Min. (g/cm^2)	Max. (g/cm^2)
Females	L- Spine	33	0.964	.191	.984	.509	1.493
	Total Hip	33	0.906	.139	.670	.546	1.216
Males	L- Spine	20	1.040	.137	.516	.877	1.393
	Total Hip	20	1.035	.122	.474	.818	1.292

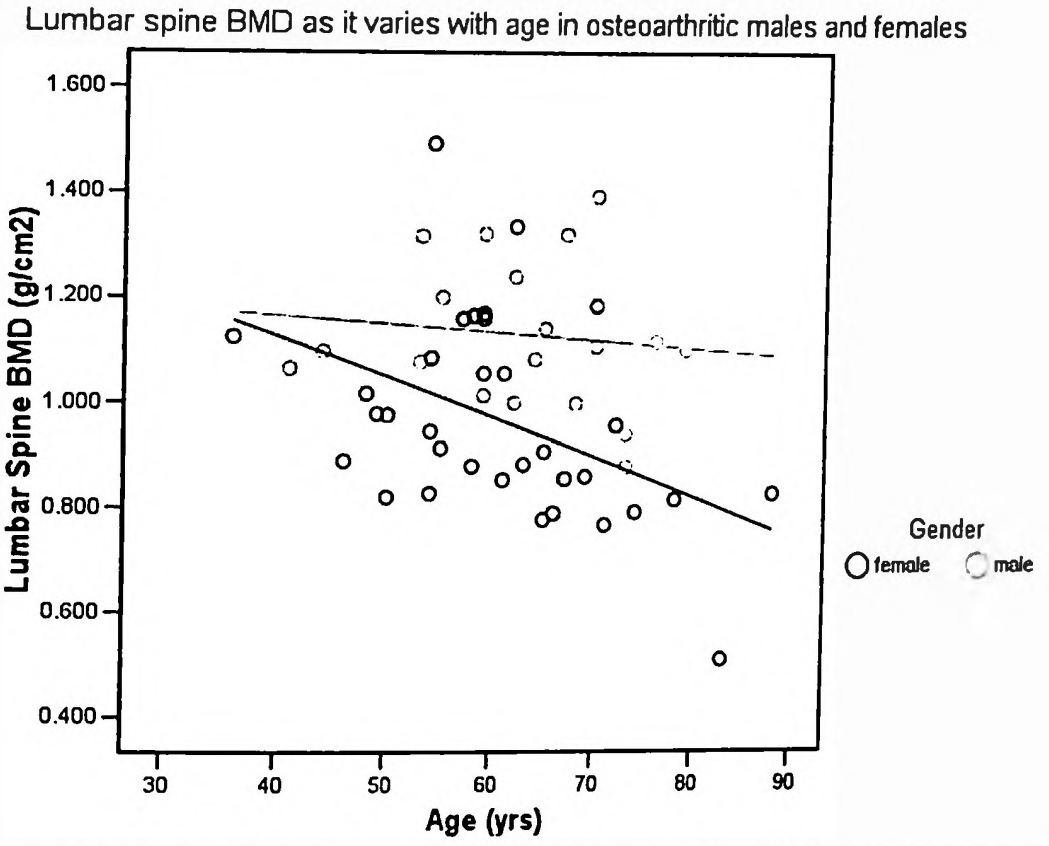


Figure 25: The relationship between total L-spine BMD and age in those with knee OA

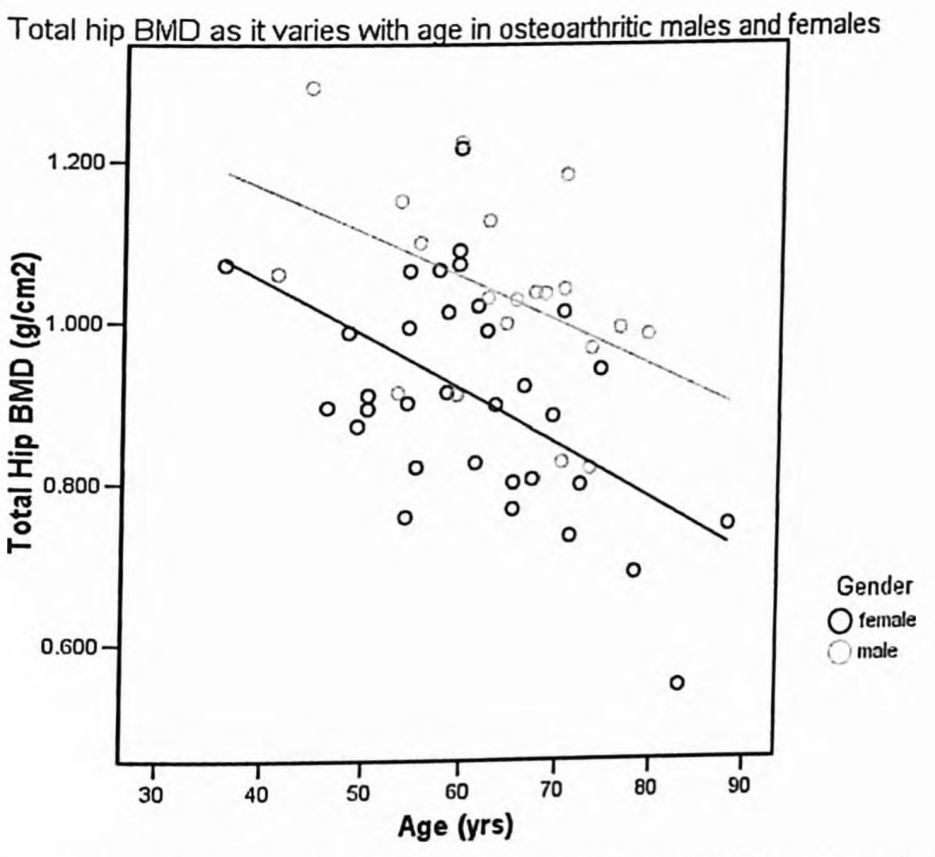


Figure 26: The relationship between total hip BMD and age in those with knee OA

Table 12: Relationships between BMI and age and BMD in the L-spine and hip in those with knee OA. β-coefficients for regression analyses are shown

	Variable	L-Spine	Total hip
Females	BMI	0.333	0.413
	Age	-0.478	-0.587
Males	BMI	*	*
	Age	*	-0.466

* = β-coefficient not significant

Table 13: Bone mineral density values in osteoarthritic individuals

	Region	N	Mean (g/cm^2)	SD (g/cm^2)	Range (g/cm^2)	Min. (g/cm^2)	Max. (g/cm^2)
Females	F- F4	32	.945	.156	.591	.686	1.277
	F- Total	32	.943	.153	.649	.543	1.192
	T- T1	31	.877	.161	.682	.514	1.196
	T- Total	31	.909	.156	.576	.570	1.146
Males	F- F4	20	.978	.104	.352	.804	1.156
	F-Total	20	1.085	.078	.306	.889	1.195
	T-T1	20	.928	.255	1.284	.448	1.732
	T-Total	20	1.030	.258	1.330	.557	1.887

F = Femur, T = Tibia

Table 14: Relationships between BMI and age and distal femur and proximal tibia BMD in those with knee OA. β-coefficients for regression analyses are shown.

	Variable	Femur		Tibia	
		F4	Total	T1	Total
Females	BMI	0.407	0.389	0.561	0.629
	Age	ns	-0.520	-0.620	-0.542
Males	BMI	*	*	*	*
	Age	*	*	*	*

* = β-coefficient not significant

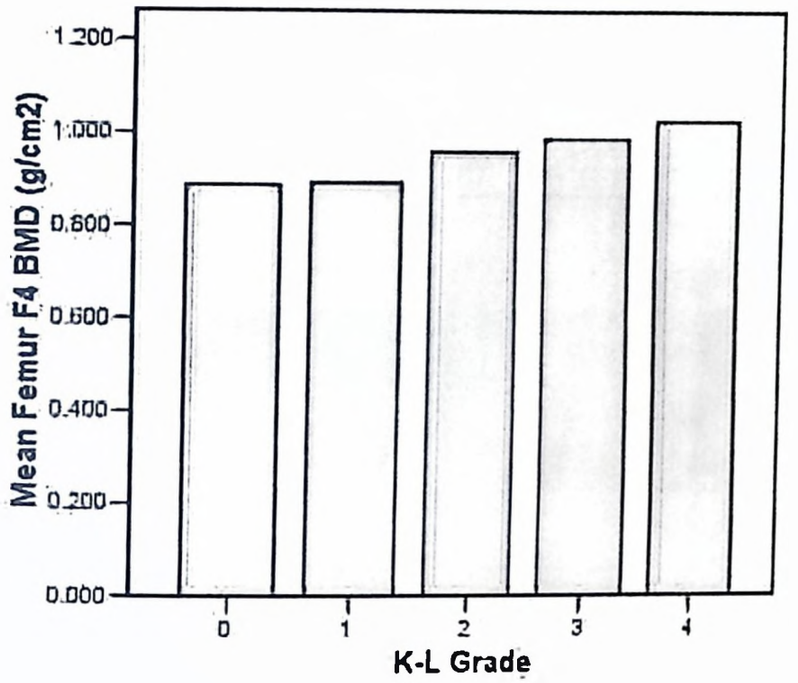


Figure 27: The relationship between BMD in the subchondral region of the distal femur and K-L grade in participants with knee OA

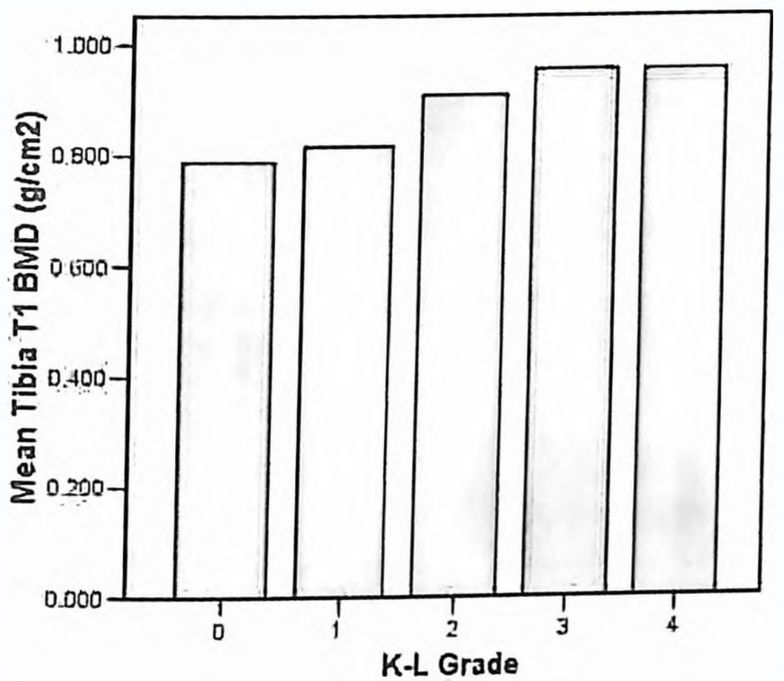


Figure 28: The relationship between BMD in the subchondral region of the proximal tibia and K-L grade in participants with knee OA

Table 15: Interobserver reliability for cartilage volume and thickness

Variable	Intra-class Correlation		Coefficient of Variation
	Coefficient	95% CI	
Med. Tib. Volume	0.772	0.293, 0.913	17.4%
Med. Tib. thickness	0.767	0.112, 0.922	17.1%

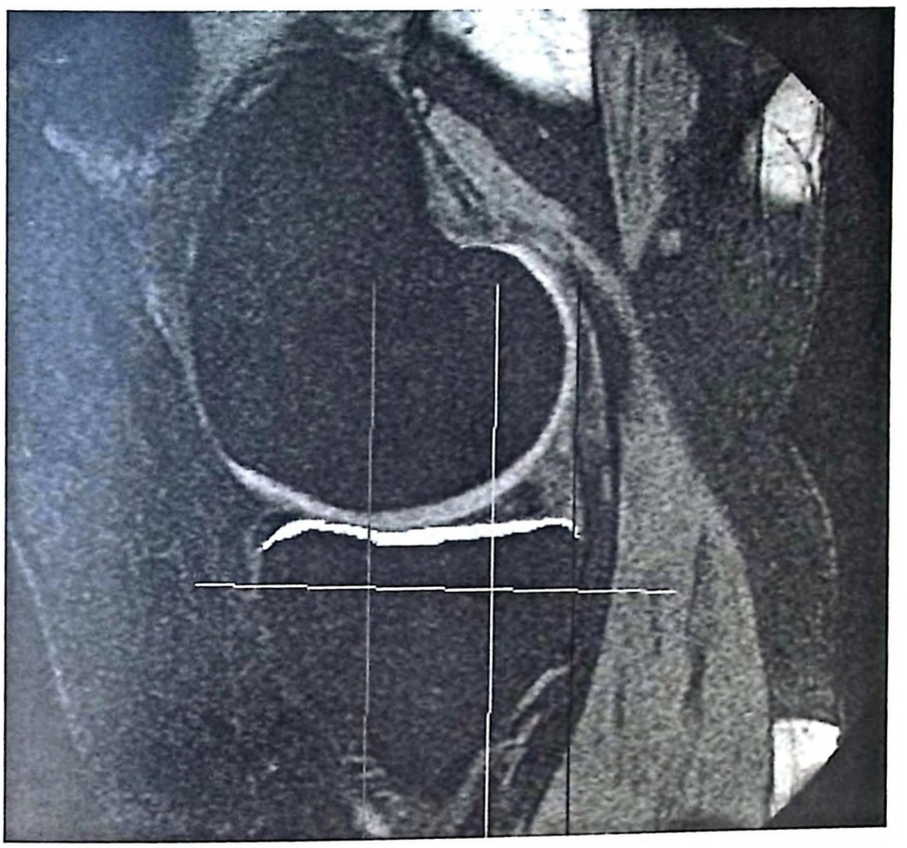


Figure 29: A slice of a saggital scan displaying the segmented articular cartilage in the medial tibia of a healthy individual

Table 16: Descriptive statistics of medial tibial cartilage volume and thickness in healthy individuals

	Variable	N	Mean	SD	Min.	Max.
Females	cartilage volume (mL)	50	1.53	0.26	0.99	2.40
	cartilage volume normalized to bone size (mL)	50	1.50	0.19	1.13	2.14
	cartilage thickness over total subchondral bone area (mm)	50	1.45	0.18	1.10	2.05
Males	cartilage volume (mL)	36	2.33	0.47	1.57	3.64
	cartilage volume (normalized to bone size (mL)	36	1.78	0.24	1.26	2.22
	cartilage thickness over total subchondral bone area (mm)	36	1.72	0.24	1.22	2.24

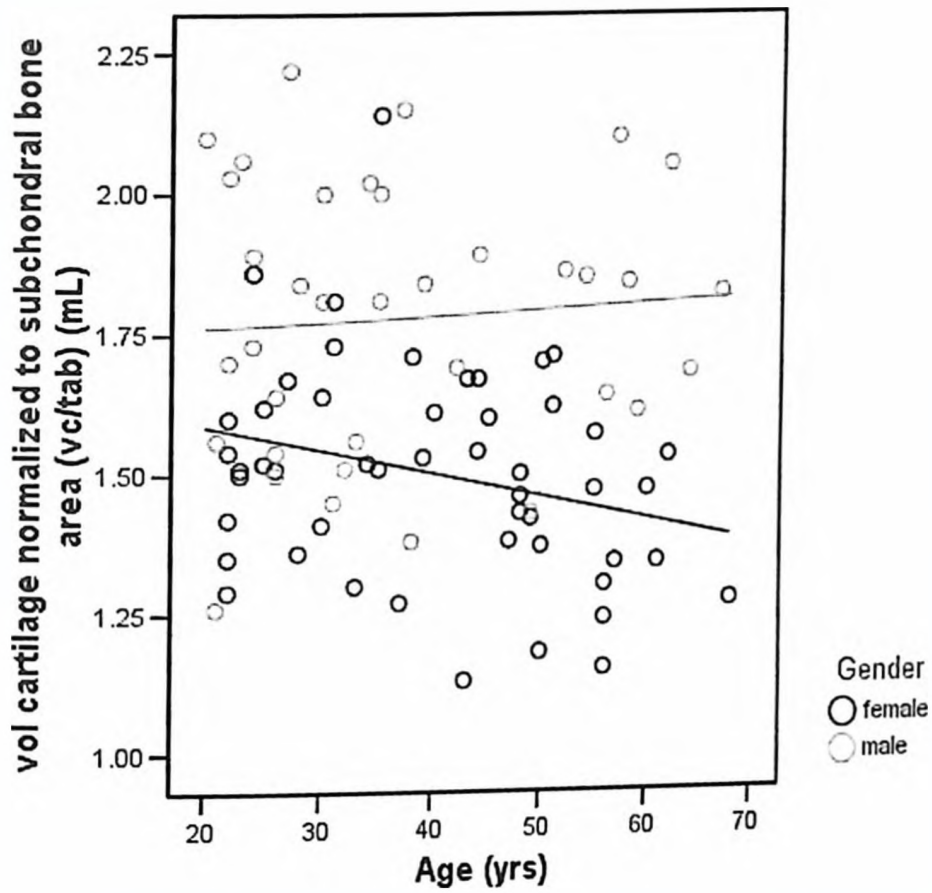


Figure 30: Relationship between cartilage volume (normalized to bone area) and age in healthy individuals

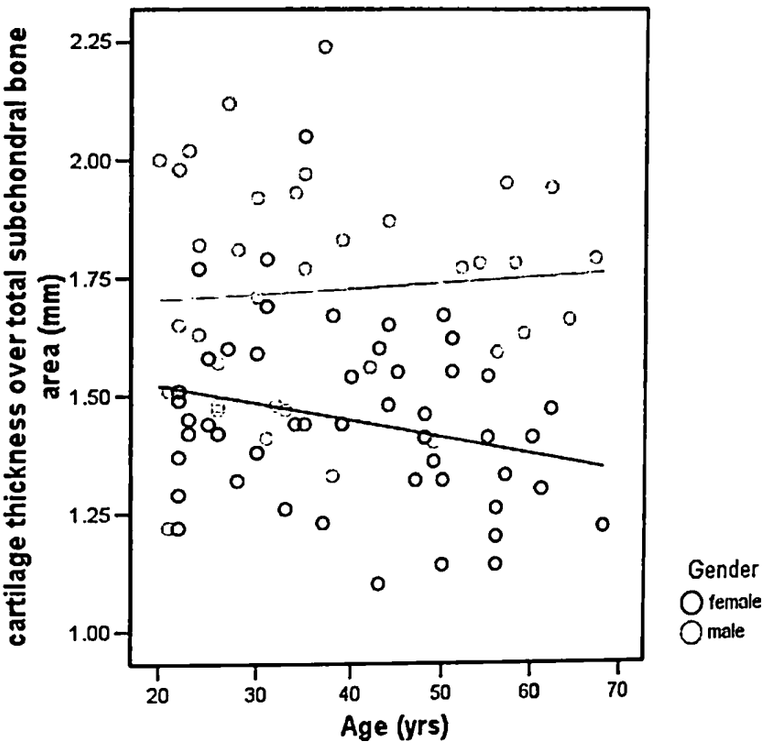


Figure 31: Relationship between thickness of cartilage and age in healthy individuals

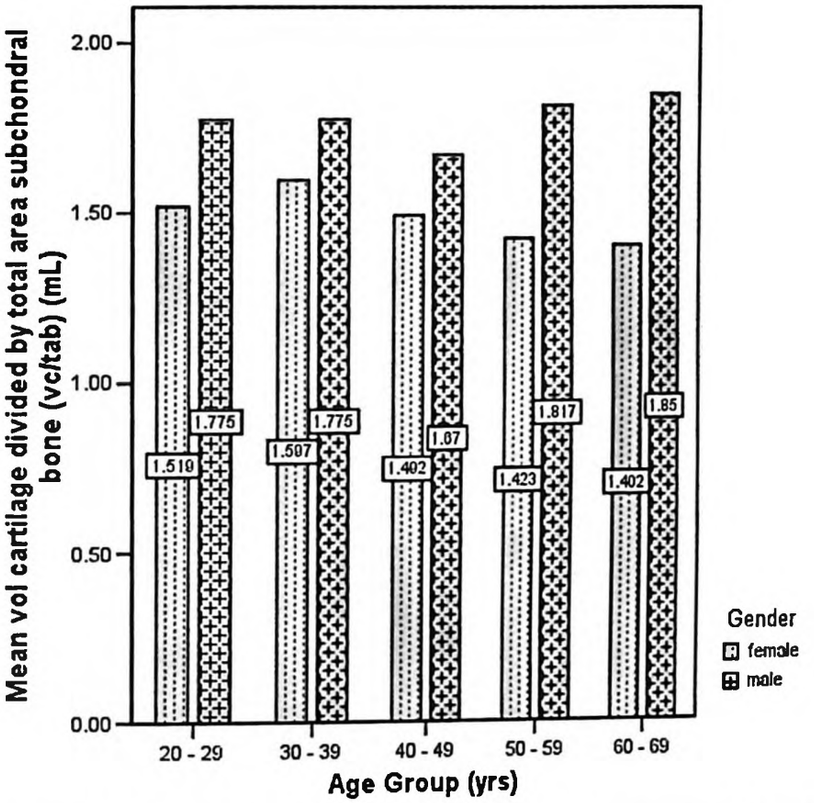


Figure 32: Mean volume of cartilage per age group in healthy individuals

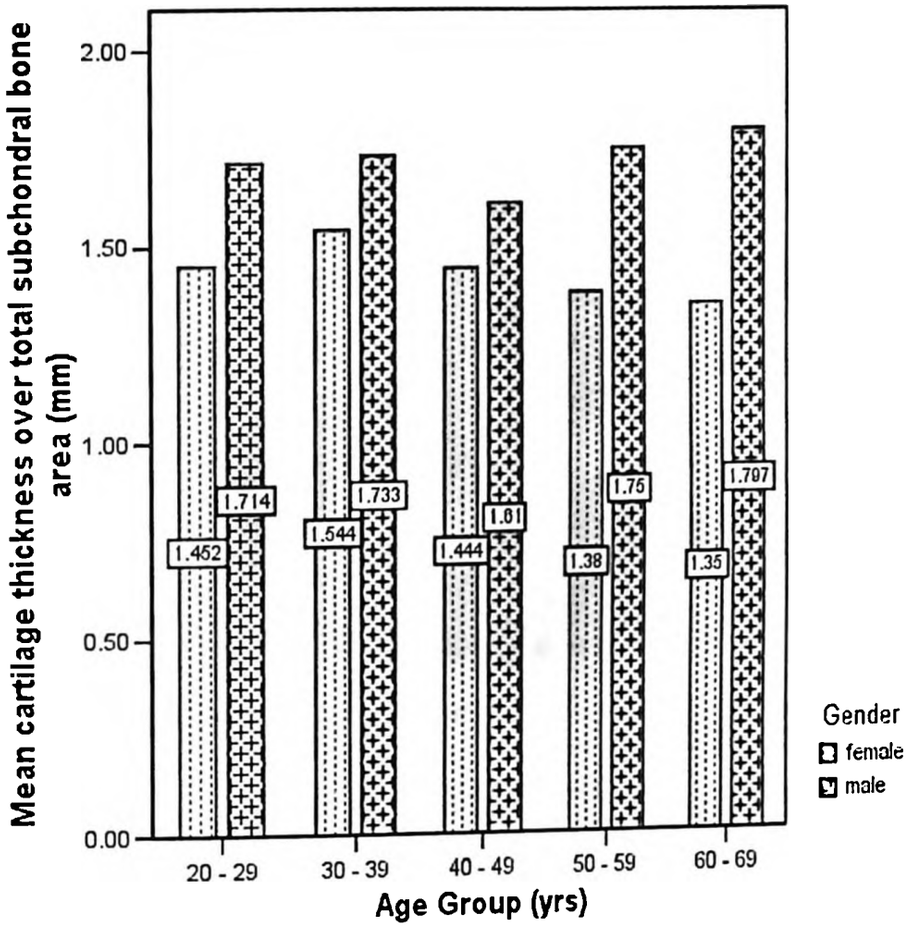
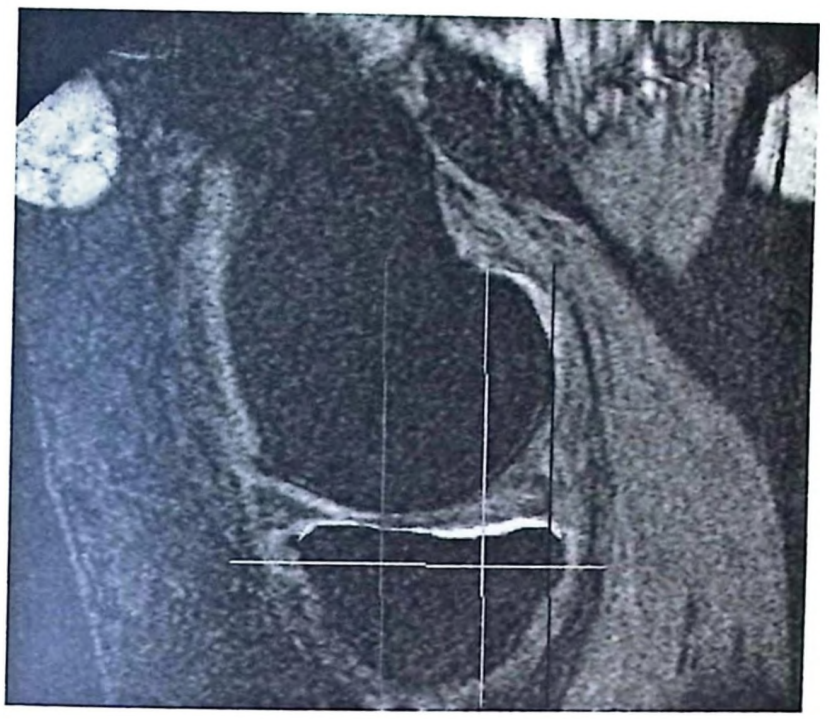


Figure 33: Mean cartilage thickness per age group in healthy individuals



Figures 34: A sagittal slice of an analyzed MR scan displaying a denuded area segmented articular cartilage in the medial tibia in an osteoarthritic participant. The denuded cartilage is shown in the middle of the tibia.

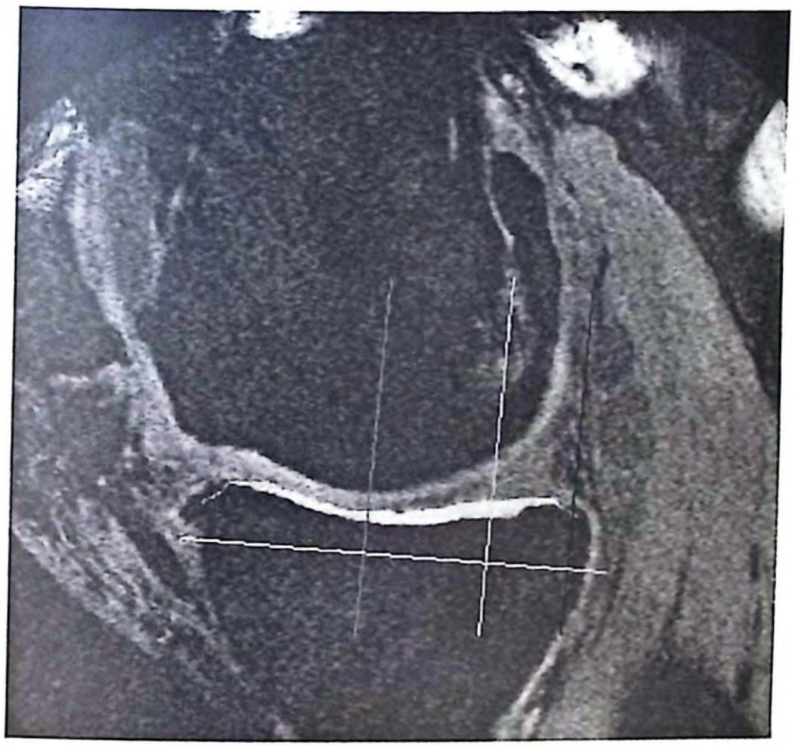


Figure 35: A sagittal single slice of an analyzed MR scan displaying segmented articular cartilage in the medial tibia of osteoarthritic participant. Osteophytes are present on the anterior and posterior aspects of the tibia, although no areas of denuded cartilage are evident.

Table 17: Descriptive statistics of cartilage volume and thickness parameters in individuals with knee OA

	Variable	N	Mean	SD	Range	Min.	Max.
Females	% bone covered with cartilage	32	87.97	22.12	78.00	22.00	100
	% bone area denuded	32	12.0	22.1	78.0	0	78.0
	cartilage volume (mL)	32	1.3	0.4	1.7	0.4	2.1
	cartilage volume normalized to bone area (mL)	32	1.2	0.4	1.4	0.4	1.8
	cartilage thickness over bone (mm)	32	1.2	0.3	1.4	0.3	1.7
Males	% bone covered with cartilage	20	82.2	25.7	82.0	18.0	100
	% bone area denuded	20	17.8	25.7	82.0	0	82.0
	cartilage volume (mL)	20	1.9	0.8	3.0	0.5	3.4
	cartilage volume normalized to bone area (mL)	20	1.4	0.5	2.0	0.3	2.4
	cartilage thickness over bone (mm)	20	1.3	0.5	2.0	0.3	2.3

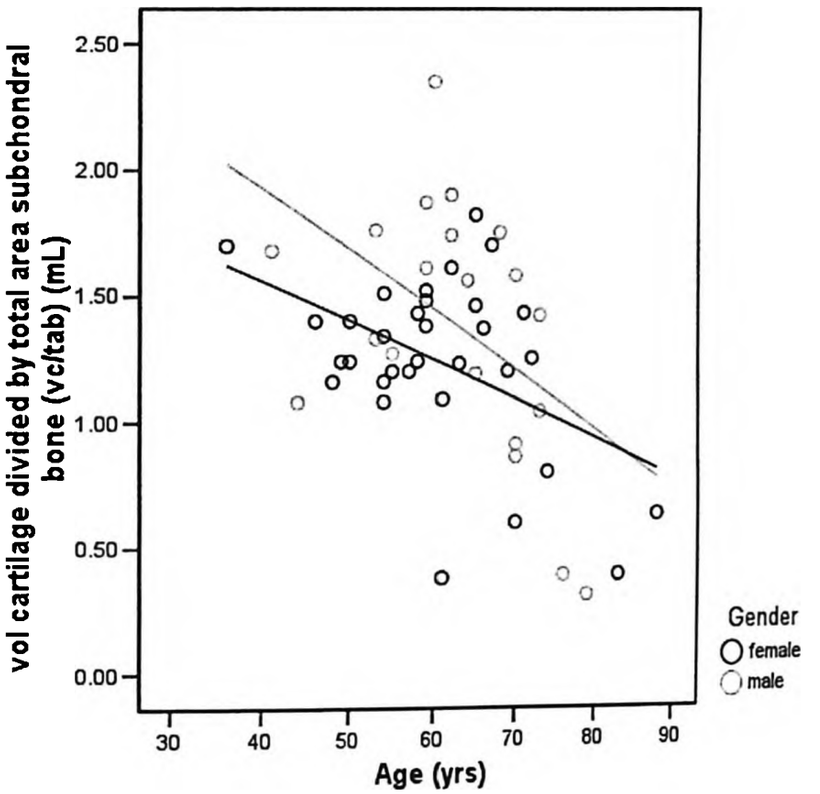


Figure 36: Relationship between cartilage volume and age in osteoarthritic individuals

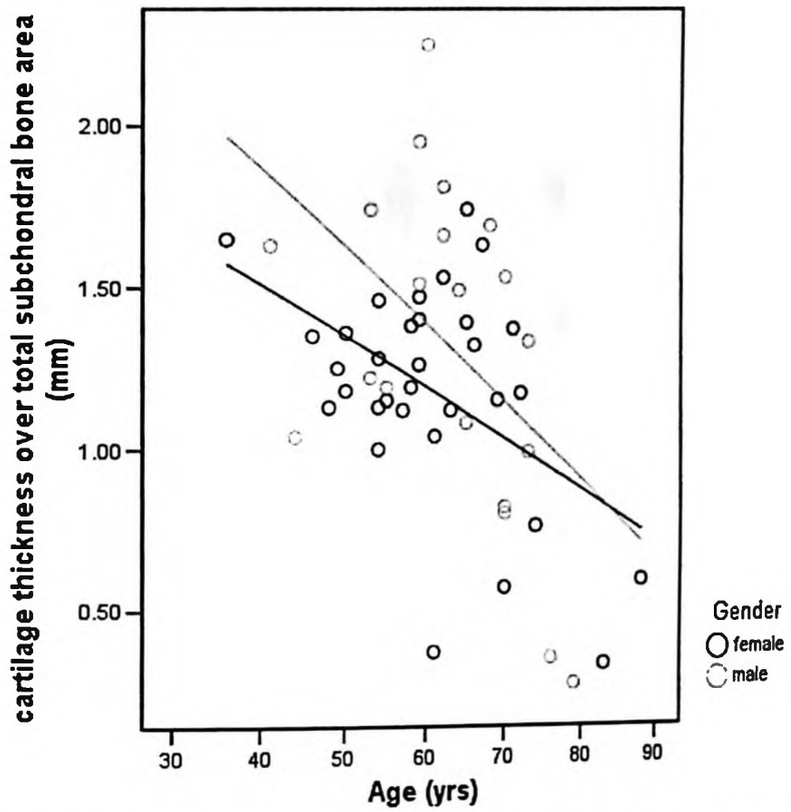


Figure 37: Relationship between cartilage thickness and age in osteoarthritic individuals

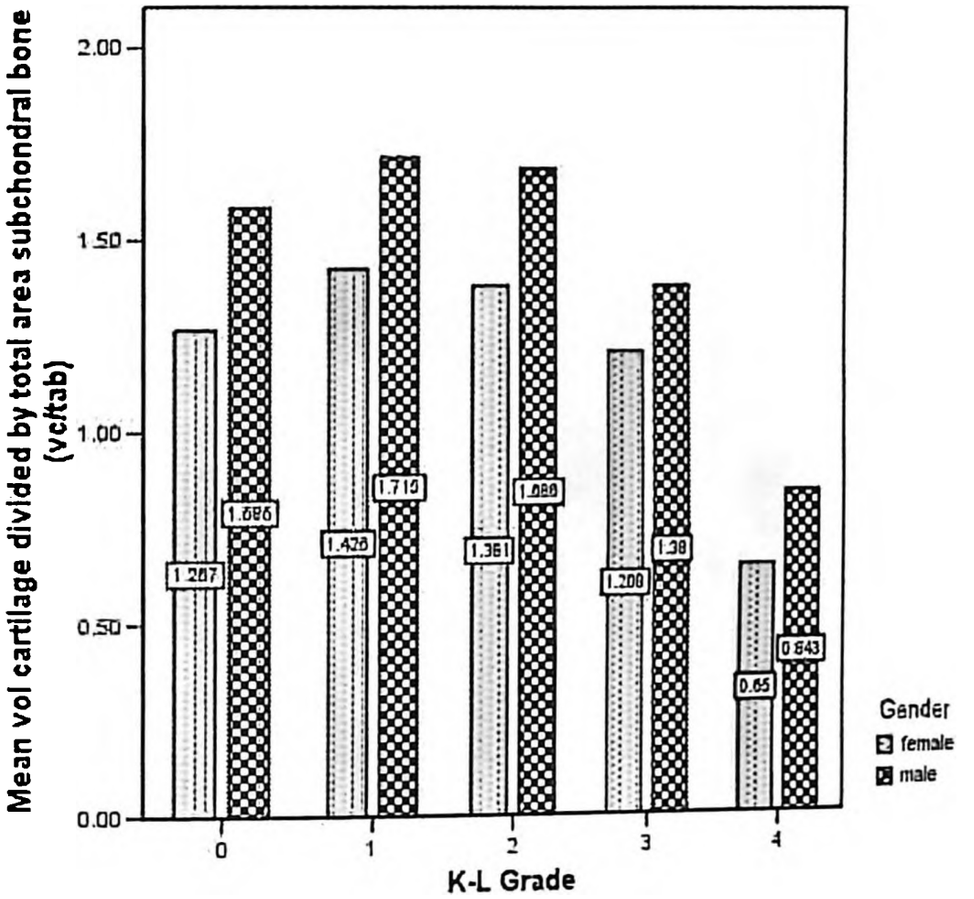


Figure 38: Relationship between medial tibial cartilage volume and disease severity in osteoarthritic individuals

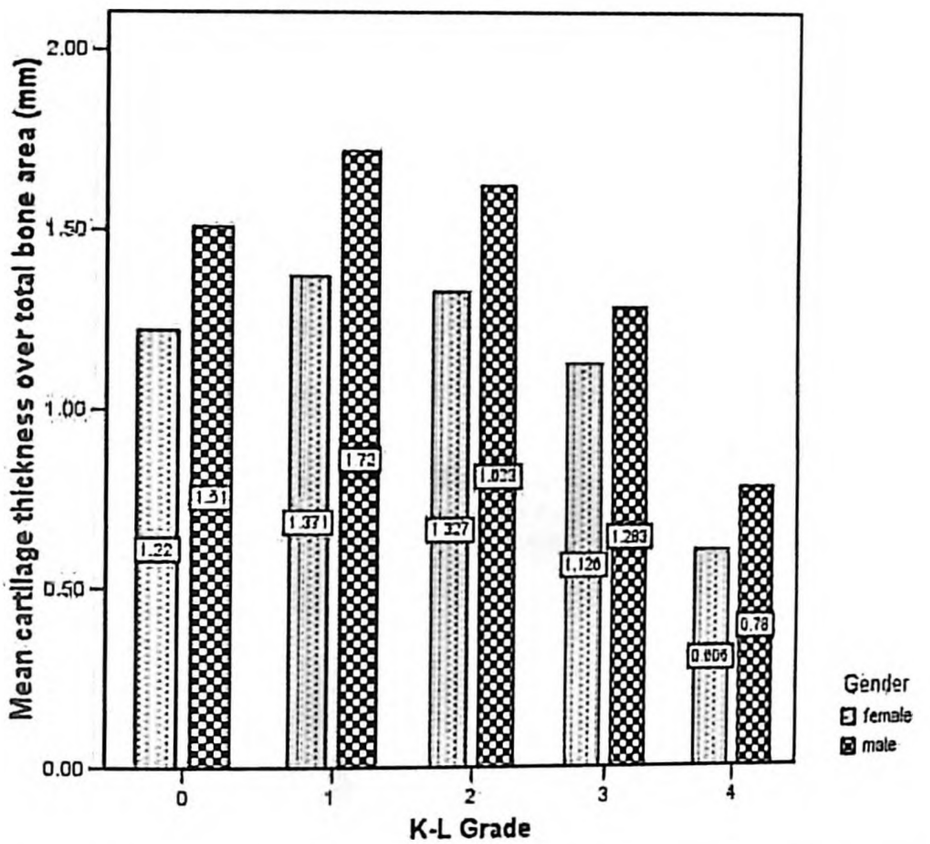


Figure 39: Relationship between medial tibial cartilage thickness and disease severity in osteoarthritic individuals

Table 18: Relationship between medial tibial cartilage volume and thickness and pain, OA symptoms from WOMAC and SF-36 questionnaires

	Cartilage Volume Correlation Coefficient	Cartilage Thickness Correlation Coefficient
Pain	-0.403 (p=0.011)	-0.496 (p=0.001)
Stiffness	-0.434 (p=0.006)	-0.469 (p=0.003)
Functional Ability	-0.484 (p=0.002)	-0.604 (p=0.001)
Total WOMAC	-0.481 (p=0.002)	-0.592 (p=0.001)
Physical Function (SF-36)	0.442 (p=0.007)	0.637 (p=0.001)

Table 19: Evaluation template for diagnostic pMRI

Grading of Soft Tissue Features Observed on pMRI	Regions Graded
A. Cartilage 0 = normal 1 = low signal intensity area extending to normal cartilage surface on adjacent images 2 = mild cartilage surface irregularity, focal defect < ½ thickness 3 = severe surface irregularity/defect > ½ thickness, < full thickness 4 = cartilage defect exposing bone	medial & lateral femur medial & lateral tibia femoral trochlea patella
B. Subchondral Cyst 0 = absent 1 = present	medial & lateral femur medial & lateral tibia patella
C. Bone Marrow Edema: 1 = none 2 = mild (<1cm) 3 = moderate (1-2cm) 4 = severe (>2cm)	medial & lateral femur medial & lateral tibia patella
D. Osteophyte: 0 = none 1 = present <0.5 cm in length 2 = present >0.5 cm in length	medial & lateral femur medial & lateral tibia femoral trochlea patella tibial spine
E. Meniscus 0 = normal; 1 = degeneration (intrasubstance high signal intensity) 2= tear (high signal intensity extending to articular surface)	medial anterior horn medial posterior horn lateral anterior horn lateral posterior horn
F. Ligaments 0 = no tear 1 = tear	anterior cruciate ligament posterior cruciate ligament patellar tendon

Table 20: Prevalence of cartilage lesions and osteophytes in different regions of the knee

Region of Interest	% of Individuals with Cartilage Lesion	% of Individuals with Osteophyte
Medial Femur	7	5
Lateral Femur	2	2
Medial Tibia	2	2
Lateral Tibia	5	2
Femoral Trochlea	9	11
Patella	7	9
Tibial Spine	N/A	18

Table 21: Prevalence of varying degrees of meniscal defects as identified per region

Region of Lesion	Lesion Grade	% of Individuals
Medial Anterior Horn (MAH)	0	30
	1	70
Medial Posterior Horn (MPH)	0	11
	1	68
	2	21
Lateral Anterior Horn (LAH)	0	32
	1	64
	2	4
Lateral Posterior Horn (LPH)	0	43
	1	52
	2	5

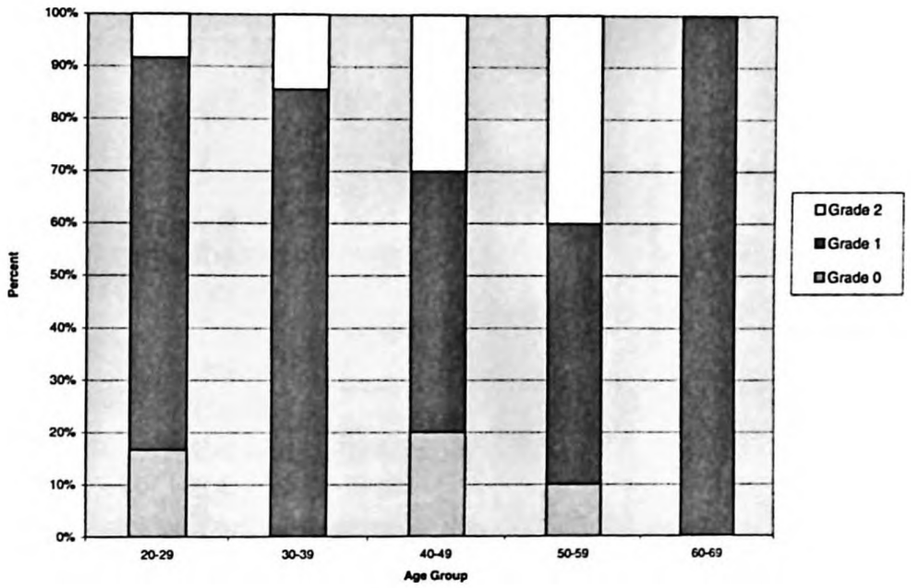


Figure 40: Grading scores of posterior horn of medial meniscus in different age groups

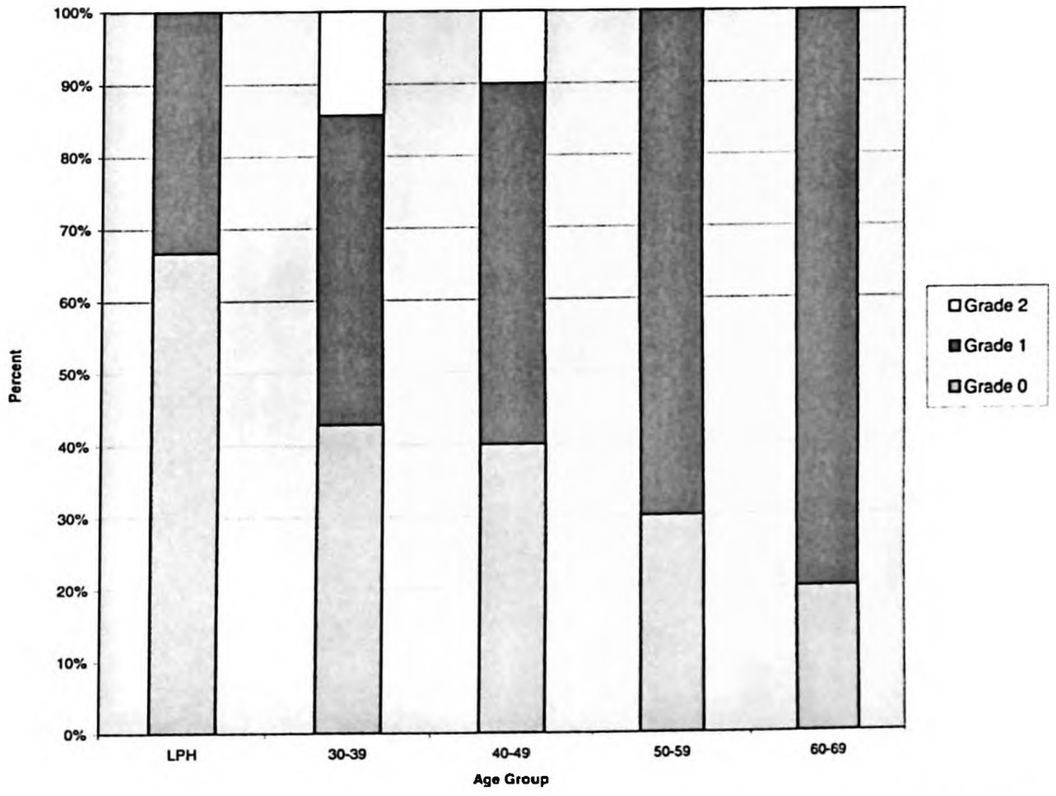


Figure 41: Grading scores of posterior horn of lateral meniscus in different age groups

Table 22: The correlation between cartilage volume thickness in the medial tibia and mJSW in the medial compartment of the knee in healthy individuals

	Variable	Pearson Correlation Coefficient (r)	95% Confidence Interval of r	r^2
Females (N=48)	Cartilage thickness over bone area	0.548	0.313-0.720	0.300
	Total cartilage volume	0.513	0.268-0.695	0.263
Males (N=34)	Cartilage thickness over total bone area	0.624	0.363-0.794	0.389
	Total cartilage volume	0.581	0.303-0.768	0.338
Together (N=82)	Cartilage thickness over total bone area	0.686	0.552-0.785	0.470
	Total cartilage volume	0.666	0.525-0.771	0.444

All correlation coefficients are significant ($p<0.05$)

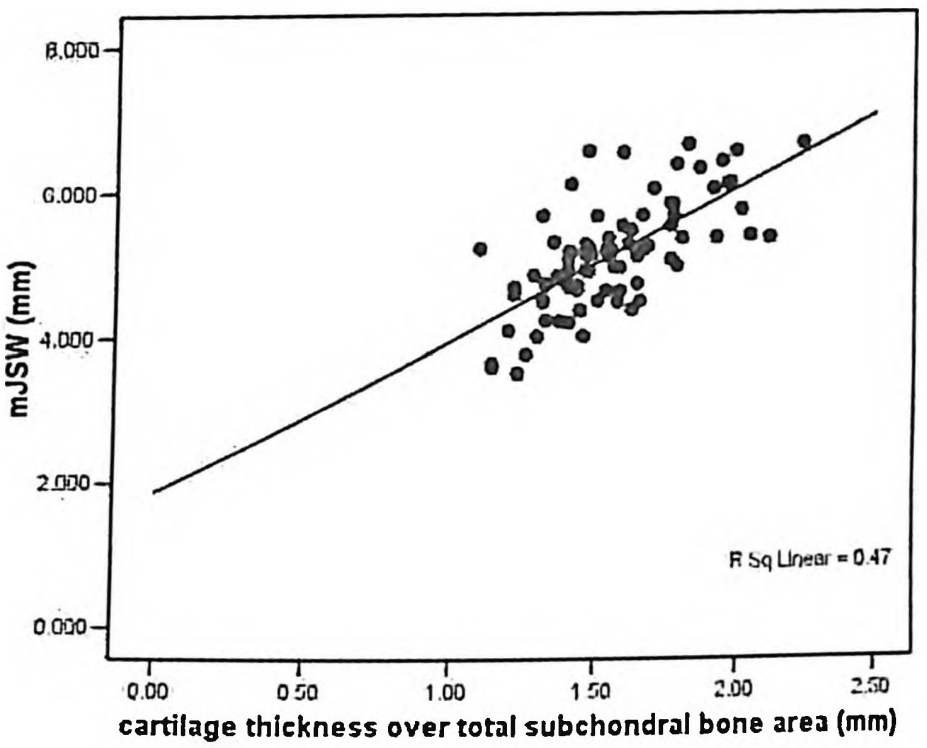


Figure 42: The correlation between medial mJSW and mean cartilage thickness in the medial tibia in healthy participants

Table 23: The correlation between cartilage volume thickness in the medial tibia and mJSW in the medial compartment of the knee in participants with knee OA

	Variable	Pearson Correlation Coefficient (r)	95% Confidence Interval of r	r^2
Females (N=32)	Cartilage thickness over bone area	0.725	0.504-0.857	0.526
	Total cartilage volume	0.630	0.361-0.802	0.397
Males (N=20)	Cartilage thickness over bone area	0.880	0.717-0.951	0.774
	Total cartilage volume	0.756	0.472-0.898	0.572
Together (N=52)	Cartilage thickness over bone area	0.800	0.675-0.880	0.641
	Total cartilage volume	0.606	0.400-0.754	0.367

All correlation coefficients are significant ($p<0.05$)

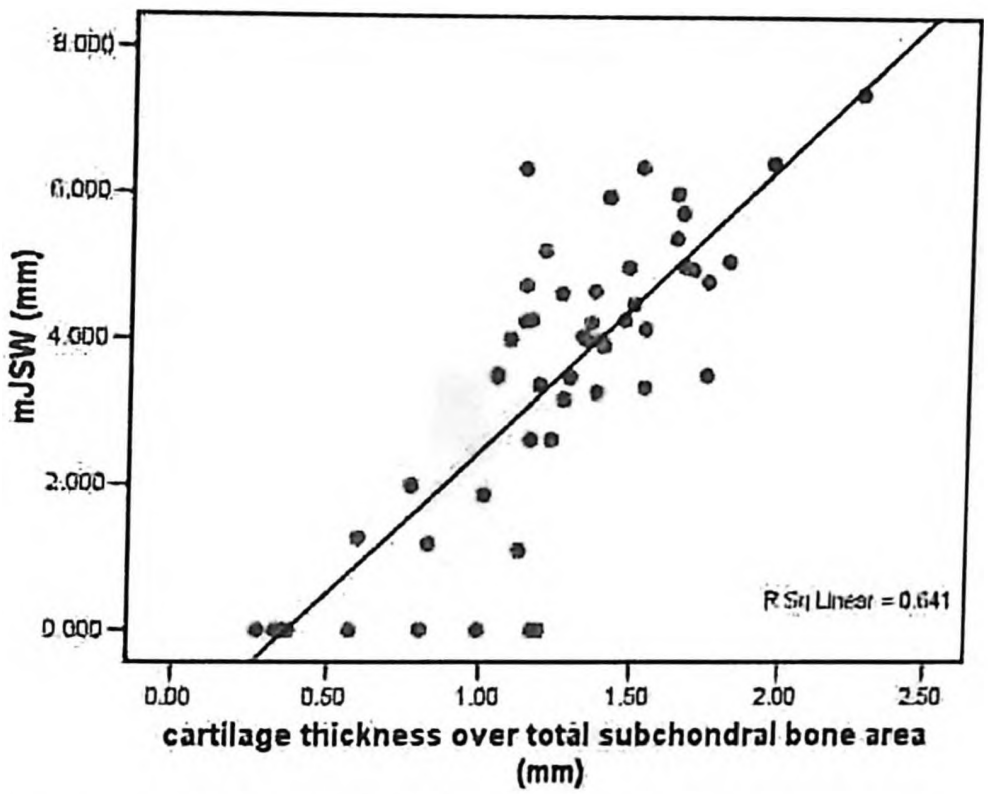


Figure 43: The correlation between medial mJSW and mean cartilage thickness in the medial tibia in osteoarthritic participants

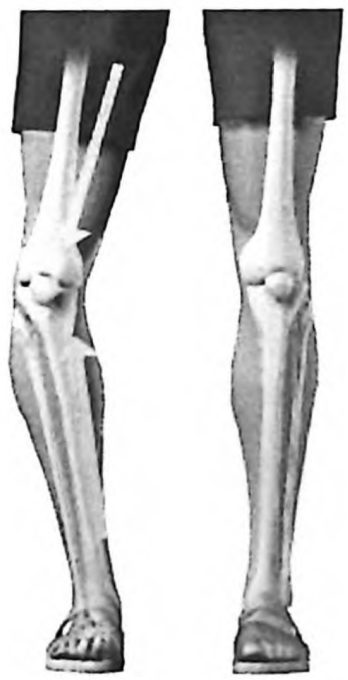


Figure 44: Schematic representation of a varus aligned knee (left) representative of tibiofemoral OA in the medial compartment

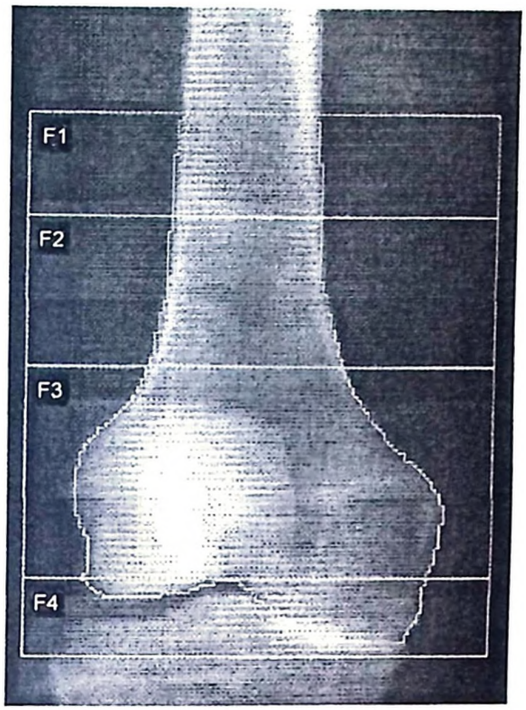


Figure 45: DXA scan of the distal femur in a patient with severe knee OA

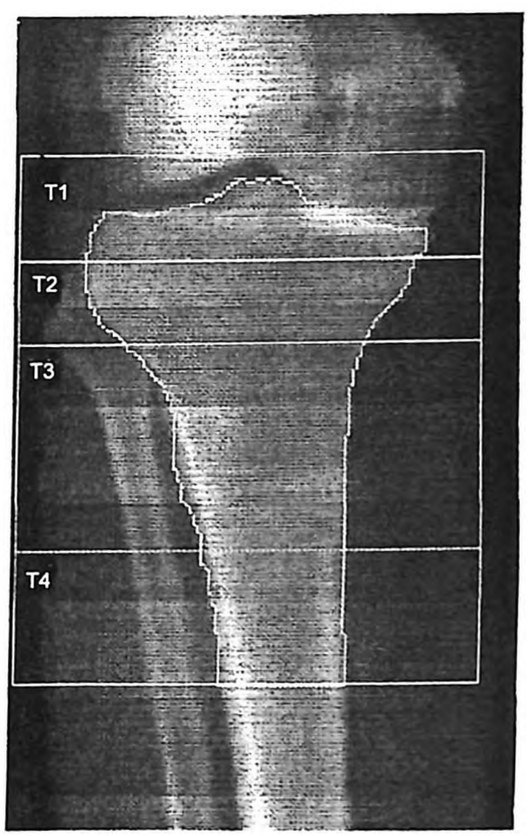


Figure 46: DXA scan of the proximal tibia in a patient with severe knee OA

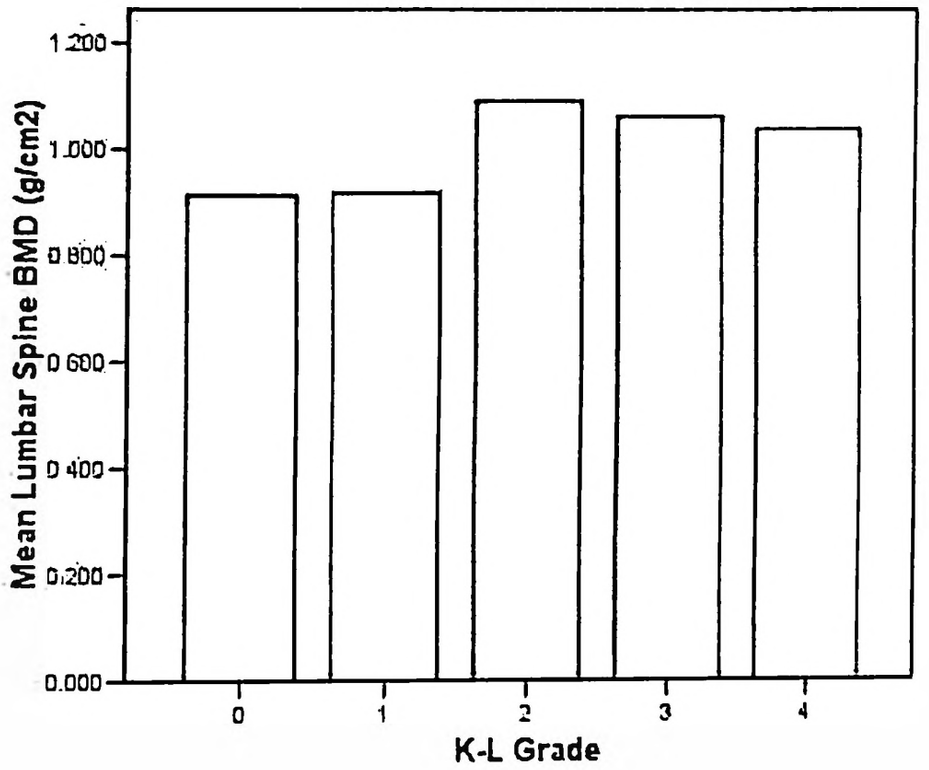


Figure 47: Relationship between K-L grade of OA and BMD in the spine in those with knee OA

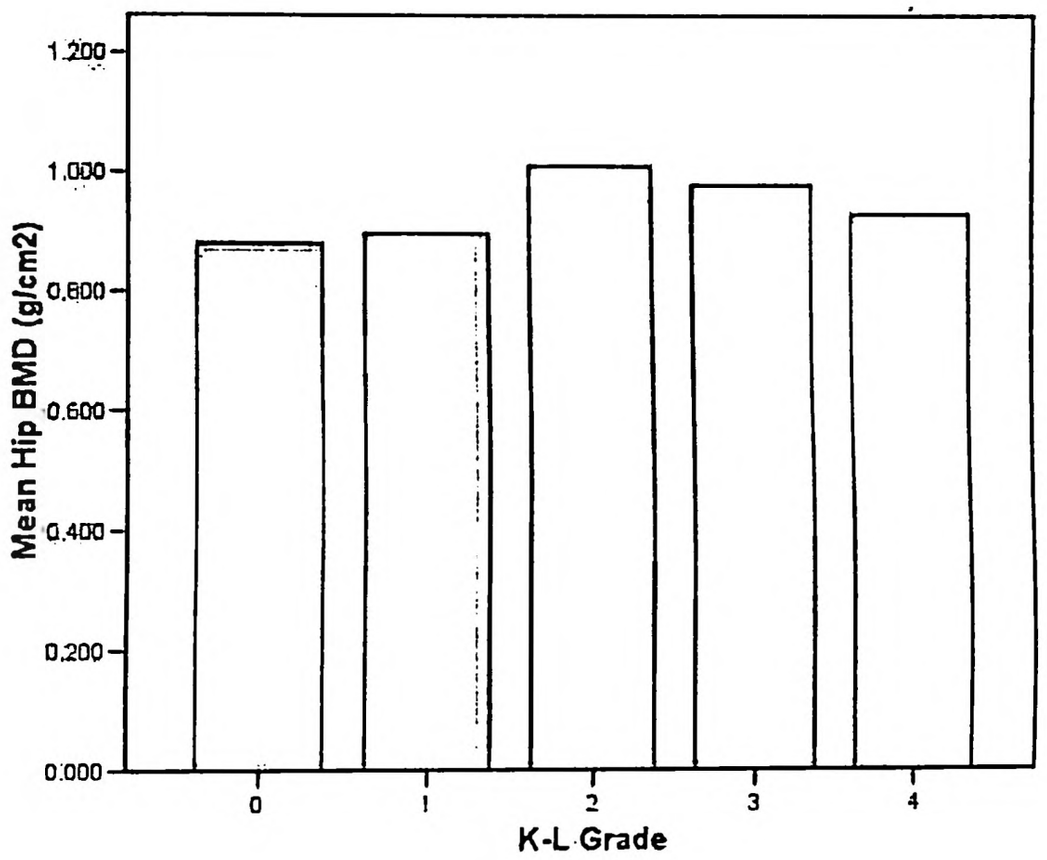


Figure 48: Relationship between K-L grade of OA and BMD in the hip in those with knee OA