The relationship between dynamic knee joint load and matrix metalloproteinases in people with and without knee osteoarthritis

by

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A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE STUDIES

(Rehabilitation Sciences)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

June 2013

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Abstract

Introduction: Knee osteoarthritis (OA) is a destructive joint disease resulting from a number of factors including excessive and repetitive loading of the joints. Even though OA symptoms begin mostly in midlife, the underlying degenerative changes of articular cartilage take place a long time before the onset, so it is important to track osteoarthritic changes at earlier phases. Currently radiology imaging is widely used for this purpose; however, radiology does not show minute changes that occur prior to onset of OA symptoms. Due to such limitations, the investigation of molecular changes is gaining momentum in today's research. Matrix metalloproteinases (MMPs) are degenerative enzymes of connective tissue and their quantities are thought to be related to OA changes. Our goal was to investigate how MMP variance is explained by OA–related factors, mainly dynamic knee joint loading.

Methods: A cross-sectional design was used to collect data on the intraarticular knee joint load, reflected by knee adduction moment (KAM), as well as serum samples in 28 participants of which half had mild to severe OA and the other half were free of OA. Laboratory-based motion analysis was used to obtain information about the KAM, while MMP levels (MMPs- 1, 3, 13) were measured using enzyme-linked immunosorbent assay (ELISA). Multiple regression analysis was used to investigate the explanatory role of KAM, and potential confounders such as age, and OA severity in explaining the variance of MMP.

Results: KAM impulse predicted significant variance in MMP-3 levels (R^2 =0.197, p=0.018). After controlling for the effect of age and OA severity, the explanatory role of

ii

KAM impulse was decreased ($R^2=0.157$), still remaining statistically significant (p=0.036). The explained variance in MMP-1, 13 levels did not reveal statistical significance from any explanatory variable.

Conclusion: This research provides evidence of a positive relationship between MMP-3 and intraarticular knee joint load, as quantified by the KAM. The relationship remained significant after controlling for age and OA severity. Our findings support the notion that MMP-3 may be a candidate for OA investigations. Since MMP levels are influenced by a number of different factors, it seems logical to consider the levels of other biomarkers along with them.

Preface

This thesis contains the work of a research study conducted by Mohammadreza Bahar under the supervision of Dr. Michael Hunt with guidance from Dr. Aziz Ghahary, and Dr. Alex Scott. The study design, data analysis, and writing the manuscript were primarily the work of the candidate. A selection of work from this thesis will be submitted for publication in peer-reviewed journals.

Ethical approval for this research study was provided by the University of British Columbia Clinical Research Ethics Board on October 17, 2011. An amendment to a previous study was approved to conduct this study. The research ethics number is H10-03092.

Table of Contents

| Abstract ii |
|---|
| Prefaceiv |
| Table of Contentsv |
| List of Tablesviii |
| List of Figuresix |
| List of Abbreviationsx |
| Acknowledgmentsxii |
| Dedicationxiii |
| Chapter 1: Introduction and Background1 |
| 1.1 What is Osteoarthritis? 1 |
| 1.1.1 Osteoarthritis prevalence 2 |
| 1.1.2 Economic burdens associated with osteoarthritis 2 |
| 1.1.3 Osteoarthritis risk factors |
| 1.1.3.1 Age |
| 1.1.3.2 Joint load |
| 1.1.4 Current tools of evaluation of osteoarthritis 4 |
| 1.2 Joint Cartilage |
| 1.2.1 Articular cartilage structure6 |
| 1.2.2 Articular cartilage histology 8 |
| 1.2.2.1 Different zones of articular cartilage |
| 1.2.3 Function of the articular cartilage10 |
| 1.2.4 Changes of cartilage in osteoarthritis11 |
| 1.2.5 Biomarkers in osteoarthritis12 |
| 1.2.5.1 Matrix metalloproteinases |
| V |

| 1.2.5.1.1 MMP-313 |
|---|
| 1.2.5.1.2 MMP-1 and MMP-1314 |
| 1.3 Matrix Metalloproteinases and Load15 |
| 1.3.1 MMP-3 and load15 |
| 1.3.2 MMP-1, -13 and load18 |
| 1.4 Measurement of Dynamic Knee Joint Load in Humans20 |
| 1.4.1 Measuring the knee adduction moment21 |
| 1.4.1.1 Peak knee adduction moment21 |
| 1.4.1.2 Knee adduction moment impulse22 |
| 1.4.1.3 Knee adduction moment units23 |
| 1.4.2 Knee adduction moment and osteoarthritis24 |
| 1.4.2.1 The relationship between knee adduction moment and osteoarthritis outcomes.24 |
| 1.4.2.2 Knee adduction moment and intra-articular knee joint load |
| 1.4.2.3 Knee adduction moment and osteoarthritis progression |
| 1.4.2.4 Knee adduction moment and osteoarthritis severity |
| 1.5 Thesis Rationale and Objectives and Hypothesis28 |
| 1.5.1 Thesis rationale28 |
| 1.5.2 Thesis objectives |
| 1.5.3 Thesis hypothesis29 |
| Chapter 2: Methods |
| 2.1 Study Participants |
| 2.1.1 Initial phone screening for study eligibility |
| 2.1.1.1 Inclusion criteria |
| 2.1.1.2 Exclusion criteria |
| 2.1.2 Radiographic screening |
| 2.2 Data Collection |

| 2.2.1 Demographic and clinical information | 36 |
|---|---------------------------|
| 2.2.2 Serum sample collection | 36 |
| 2.2.3 Biomechanical data | 37 |
| 2.3 Data Analysis | |
| 2.3.1 Serum sample analysis | |
| 2.3.2 Biomechanical data analysis | 40 |
| 2.4 Sample Size Calculation | 41 |
| 2.5 Statistical Analysis | 42 |
| Chapter 3: Results | 44 |
| 3.1 Participant Demographic and Clinical Outcomes | 44 |
| 3.2 Multiple Regression Models | 48 |
| Chapter 4: Discussion | 52 |
| 4.1 Interpretation of the Findings | 52 |
| 4.2 Study Limitations | 57 |
| 4.3 Future Directions | 60 |
| References | 63 |
| Appendix A: Knee Adduction Moment Curves | 76 |
| Appendix B: Letters of Explanation and Consent Forms | 105 |
| Appendix B1 Blood banking for exploratory future analysis from the: Contro | ols and Biomarkers 105 |
| Appendix B2 Biomarkers and knee osteoarthritis: Associations with joint lo exercise | ad and effects of 109 |
| Appendix B3 Functional mobility control database | 116 |

List of Tables

| Table 1. Kellgren and Lawrence grading scale | 35 |
|--|--------|
| Table 2. Demographic information | 45 |
| Table 3. Outcome measures | 47 |
| Table 4. Summary of regression models for predicting the matrix metalloproteinases lev | /els49 |

List of Figures

| Figure 1. Cross-sectional view of the knee joint | 8 |
|--|----|
| Figure 2. Depiction of the knee adduction moment. | 20 |
| Figure 3. Knee adduction moment impulse and peak knee adduction moment | 22 |
| Figure 4. Linear regression plots (a, b, c) | 51 |

List of Abbreviations

| AAC | Arthritis Alliance of Canada |
|---------------|--|
| BMI | body mass index |
| BW | body weight |
| cm | centimetre |
| Col-II | collagen type II |
| CRP | C - reactive protein |
| ECM | extracellular matrix |
| ELISA | enzyme-linked immunosorbent assay |
| GRF | ground reaction force |
| Ht | height |
| НТО | high tibial osteotomy |
| Hz | Hertz |
| JSN | joint space narrowing |
| КАМ | knee adduction moment |
| KAM imp | knee adduction moment impulse |
| KAM imp Total | knee adduction moment impulse total |
| kg | kilogram |
| Kg/m² | kilogram per square metre |
| K/L | Kellgren and Lawrence |
| LA | lever arm |
| MABLab | Motion Analysis and Biofeedback Laboratory |
| MMP | matrix metalloproteinase |
| MPa | mega Pascal |

| MRI | magnetic resonance imaging |
|-------|---|
| ng/ml | nanograms per millilitre |
| Nm | Newton metre |
| Nm.s | Newton metre second |
| OA | osteoarthritis |
| pg/ml | picograms per millilitre |
| SD | standard deviation |
| SPSS | Statistical Package for the Social Sciences |
| TIMP | tissue inhibitor of metalloproteinase |
| UBC | University of British Columbia |
| VGH | Vancouver General Hospital |

Acknowledgments

I would like to express my special thanks and regards to Dr. Michael Hunt who patiently guided me throughout this academic journey. His constructive feedbacks and exquisite critiques taught me a lot and I will always appreciate him for that. I give my sincere thanks to my honourable committee members Drs. Ghahary and Scott for allocating a part of their valuable time to help me in my project.

Many thanks to fabulous professors of the Rehabilitation Sciences programme, whom I had the privilege of learning from them, and my dear colleagues in MABLab and BCPFF lab for all their kind support.

Finally, I would like to thank my beloved family members for giving me their love and support and being there beside me through hard times. I dedicate this work to my parents.

Dear mother, I want you to know that you are the most precious thing I have in my life. You brought me to life, took care of me, taught me right from wrong, and gave me your love and kindness. Thank you for everything that you did and you do for me.

Dear father, it is with you that the meaning of being a true man comes to light for me. None of my achievements would have been possible without you. I will always appreciate your gracious help and support.

Chapter 1: Introduction and Background

1.1 What is Osteoarthritis?

Osteoarthritis (OA) is the most common type of the arthritic diseases and is characterized by degenerative changes of the articular cartilage that are accompanied by symptoms such as pain, stiffness, and occasional swelling of the affected joints (1-3). The joints that are most frequently affected by OA include the knee, hip, small joints of the hand, and spine (1), with the knee being the most commonly affected joint (4). Therefore, for the purposes of this thesis, the focus will be on knee OA.

OA leads to muscular weakness, functional limitation, impaired quality of life, and physical disability (5-7). Though predominantly affecting the articular cartilage, OA can also influence different tissues that are found in a joint including the subchondral bone, joint capsule tissue, and the synovium (8, 9). At the present time, no definite etiology exists for OA; however, it is thought to result from an imbalance between the synthesis and degeneration of articular cartilage, in that degeneration occurs at a greater rate than synthesis (8). This imbalance leads to cumulative changes that in the long term reveal the clinical manifestations of OA (8). There is currently no cure for OA.

1.1.1 Osteoarthritis prevalence

OA is the leading cause of chronic physical disability in the elderly (10). The Arthritis Alliance of Canada (AAC) has released a number of epidemiological reports about the prevalence and burden of OA in Canada (4, 10). It has been reported that as of 2010, 4.4 million Canadians were living with OA, and if no changes in the current patterns of progression are made, more than 10 million Canadians (or 25% of the Canadian population) will be affected with OA by 2040. It is estimated that of the 4.4 million Canadians currently affected by OA, about 500,000 Canadians suffer from moderate to severe disability (4).

1.1.2 Economic burdens associated with osteoarthritis

The increasing burden imposed by OA on the economy of Canada is devastating (4). Numbers that represent the costs of surgeries and hospitalization, frequency of clinician visits, and the working hours lost due to OA appear to be increasing (4). In the AAC report, it has been indicated that over 40% of Canadians in the labour force will develop OA later in life (10). In the year 2010, the direct cost of OA was reported to be \$10.2 billion. Statisticians suggest that if no changes are made to alter the current trend of OA management, this amount will increase to \$17.3 billion annually by 2040 (4).

1.1.3 Osteoarthritis risk factors

In the literature, a wide variety of risk factors have been attributed to the development and progression of OA. Two of the most common risk factors for OA include age and excessive loading.

1.1.3.1 Age

OA can be defined as an age-related change or disease in the human body (8). It has been repeatedly mentioned in the literature that the age of individuals is one of the most important risk factors in OA; that is the older a person gets, the higher the chance of osteoarthritis occurring in that person (8, 11). Chondrocytes, the only cells that are found in healthy cartilage, are exposed to elevated stress during aging (3). This stress is accompanied by changes in the content and the structural harmony between the joint components (3). Woolf suggests that the prevalence of OA increases with age because of the irreversible changes that take place inside the cartilage during the process of aging (2). These changes can lead to the highest incidence of the disease after the sixth decade of life (8). Silman and Hochberg reported in 1993 that the prevalence of OA is slightly higher in men at ages younger than 45, while more women over the age of 45 years are affected with OA compared to men of the same age range (12).

1.1.3.2 Joint load

It is generally agreed that increased load on a joint is one of the mechanisms that leads to the breakdown of articular cartilage within the knee (13-16). Excessive loading

can be applied to a joint in the form of a trauma or obesity (11), or through occupations or activities involving repetitive and intensive loading of the knees (17). Various epidemiologic studies conducted in different populations have suggested that excessive loading is highly associated with the development and progression of OA, especially in joints that undergo excessive loading on a consistent basis (3, 11, 13, 17, 18). On the other hand, many investigators have tried to manifest the role and importance of load in the development of OA within clinical contexts. Andiacchi et al. demonstrated that degeneration takes place mostly in the medial compartments of the knee that can be more clearly observed in bow-legged individuals, resulting in biological changes (19). This occurs because of the excessive load that is applied more to the medial compartment of the knee in comparison to the lateral compartment during movement. Additional evidence of the effect of load on articular cartilage breakdown has been demonstrated by Radin et al. They found that adult rabbits subjected to impulsive loading of the knee joints developed changes in the joints consistent with those of degenerative joint disease (20). In another study, Radin et al. demonstrated that by applying repetitive external forces to the bones of cows, in vitro, their joint cartilage breaks down and releases cartilage molecules into the experimental buffer (21). These results appear to support the notion that high joint loads lead to cartilage degeneration indicative of OA.

1.1.4 Current tools of evaluation of osteoarthritis

Along with clinical evaluations of OA that take different manifestations of the disease into account, different methods of imaging techniques are also used for

evaluation and detection of OA. The diagnosis made upon the outcomes of the imaging techniques is primarilly based on the degenerative changes that can be observed in the articular components of a joint (11). These changes include formation of osteophytes along joint margins, subchondral sclerosis, and narrowing of the joint spaces between two bones (11).

Currently, the most common method for detection of these changes is conventional radiography, or x-ray imaging (22). In recent years, improvements have been made in the radiographic positioning of the patients as well as in other elements of x-ray imaging protocols (23). Notwithstanding the advancements, due to the poor sensitivity and the relatively large precision error of the technique, as well as the twodimensional demonstration of the joint that it provides, this technique lacks the capability of detecting early joint degeneration and the accurate monitoring of potential therapeutic interventions (23, 24).

Magnetic resonance imaging and arthroscopy are two other evaluative techniques that are available, but not frequently used due to the expense and the invasiveness of the procedures, respectively (23). Despite the valuable information that imaging techniques can provide, there are still many shortcomings that prevent the attainment of an accurate assessment of joint health (24). Moreover, the findings obtained from these techniques are not always accompanied by clinical symptoms of OA; meaning that the clinical and radiographic features of the disease can be potentially presented independently from each other (11, 18). That being the case, for a diagnosis of OA, one cannot solely rely on the findings from the imaging techniques, and this necessitates the implementation of methods that are more sensitive to minute changes

that occur inside a joint. This need has drawn the attention of many researchers towards the study of molecular and chemical changes that occur as a result of osteoarthritic changes (22).

These biological markers, commonly referred to as biomarkers, are thought to have a potential impact on our understanding of the minute changes of the joint cartilage, and together with clinical evaluation and imaging techniques, it is hypothesized that they can provide the best means of monitoring and diagnosing OA (23). However, there is still much work to be done to establish the validity of biomarker analysis for the purposes of knee OA diagnosis and monitoring. The implementation of biomarkers as a means of evaluating OA requires a basic understanding of articular structure and function.

1.2 Joint Cartilage

1.2.1 Articular cartilage structure

As one of the members of the connective tissue family, cartilage is the flexible and jelly-like tissue that is found in various places in the body, such as the joints between bones, inter-vertebral discs, ear, nose, and bronchial tubes. Cartilage tissue is not as rigid as bony tissues; however, it is stiffer than muscle. The cartilage between bones is referred to as articular cartilage, which makes up a large domain of the anatomical joints, and will be the primary type of cartilage discussed in this thesis.

Chondrocytes are the only cells found in healthy articular cartilage. A mature, healthy cartilage is composed of two primary phases—a liquid and a solid phase. The

liquid phase is mainly composed of water that makes up 70-80% of the liquid content of the cartilage. Synovial fluid is also present in the joint capsule and contains inorganic salts. Most of the water content is found in the molecular pore space of the extracellular matrix (ECM) that fills up the space between the chondrocytes but is also spread throughout the entire articular cartilage tissue (25-27).

Twenty to 30 percent of the cartilage tissue is composed of a solid framework of structural macromolecules that give the tissue its form and function. Of this solid composition, 50-75% is made up of different collagens and 15-30% consists of proteoglycans. The remainder consists of minor protein molecules (25-27). Collagens are the principal molecules in providing the structural formation of connective tissue, including articular cartilage. In articular cartilage, collagen type II (Col-II) is the predominant collagen type, comprising more than half of the solid phase weight within the cartilage tissue (28). This protein structure is a triple helix that is composed of three Coll-2 α chains. Fibres of Coll-II construct a tri-dimensional scaffold which represents the tensile strength properties of cartilage (29).



Figure 1. Cross-sectional view of the knee joint

In anatomy, a joint is where two bones articulate. The ends that make contact with each other are covered with articular cartilage, which allows smooth movement of bones on each other by minimizing the friction between them. The joint is encapsulated inside the joint capsule that contains synovial fluid and is covered with synovial membrane [Source for figure (30)].

1.2.2 Articular cartilage histology

1.2.2.1 Different zones of articular cartilage

Cartilage is organized into successive zones including superficial or tangential, middle or transitional, deep or radial, and calcified zones. The morphology and functional features of the chondrocytes and the constituents of the ECM vary in these regions (3, 31). The superficial, or tangential, zone makes up 10-20% of the upper layer of the articular cartilage tissue. Structure of this zone is characterized by the predominant presence of collagen fibres which are situated parallel to the surface of the cartilage (31, 32). The proteoglycan content and the permeability of the matrix are fairly low in the superficial zone. The cellular conformation presents a densely packed structure as well as a flattened, discoid exhibition in the shape of the chondrocytes that are aligned with the adjacent collagen fibres (31, 33). Chondrocytes in this zone secrete lubricin, or superficial zone protein, which provides a smooth collagenous surface for the facilitated gliding motion of articular surfaces during joint movement (34).

The middle, or transitional, zone comprises about 40-60% of the articular tissue thickness. The shape of the collagen fibres in this zone is arcade-like, and the collagen molecules are spread within fibres with nonspecific orientation (31, 35). the proteoglycan content of the middle zone is the highest of all the zones of articular cartilage (36), the cellular density is noticeably lower, and the cells have more of a spherical shape (33).

The collagen structure of the deep zone is distinguished by immense fibres that form bundles positioned perpendicular to the articular surface and are attached to the subchondral bone (37). In comparison with the superficial and the middle zones, the deep zone has the lowest cellular density (33) and the proteoglycan content is much lower than the middle zone (36). The moderately elongated cells of the deep zone are usually piled together in a columnar arrangement and are oriented parallel to collagen fibres, perpendicular to the joint surface (38).

It is in the calcified zone of cartilage that transformation of cartilage into the subchondral bone takes place. The density of this region is something between the stiff

bony area and the flexible cartilage (39). Below the calcified region lies the subchondral bone that marks an end for the cartilage tissue (31).

1.2.3 Function of the articular cartilage

In physiological conditions, distribution of the loads throughout a loaded joint is managed by the articular cartilage (11). The cellular and molecular components of the cartilage give it the capability of resisting different types of loading, applied to the joint during daily life (40). The distribution of the load is performed in a manner such that minute amounts of cartilage tearing are expected to occur (25), meaning the turnover rate of cartilage is thought to be noticeably low in a way that the half-life of collagen and proteoglycans are estimated to be 100 years and 325 years, respectively (11). The fluid content of the joint plays a crucial role in the absorption of excessive loading applied to the joints. It provides a cushion-like environment within the articular cartilage that has the capability of bearing sudden and gradual loading, therefore alleviating the amounts of energy that have to be handled by the solid phase of the cartilage (31).

In addition to the mechanical properties of the fluid phase, it is also important in maintaining joint integrity. Since there is no vascularity within the cartilage tissue, chondrocytes receive their nutrients via the effusion that takes place inside the joint space. It is one of the primary roles of the interstitial fluid to provide the required nutrients of the joint as well as to transport the waste outside the joint space. Articular changes of the cartilage are presented as a result of loading that can be categorized into tensile, compressive, and shearing stresses within the tissue (25).

1.2.4 Changes of cartilage in osteoarthritis

The fluid content within articular cartilage acts as an initial and essential shockabsorber in response to different kinds of mechanical loading applied to the joint (31). When the water content of the articular cartilage is reduced as a result of chondrocyte senescence, the loading within the joint is then directly transferred to the solid content of the cartilage. The loading that is essential for maintaining the health of the cartilage can now create irreversible changes to the solid components of the ECM. After application of load onto the solid content of a joint, which primarily consists of Coll-II and proteoglycan fibres, the induced physiological responses are going to be different from the way they used to be in the presence of adequate amounts of fluid (3, 8). The result of this occurrence is expressed as molecular changes and consequent cartilage breakdown (8, 9). Since water content is typically reduced in articular cartilage during normal ageing, this likely provides a strong explanation for the higher prevalence of knee OA in the elderly population.

An osteoarthritic joint can suffer from molecular imbalances within the ECM content that can be exacerbated by further joint loading and contributing to articular cartilage degeneration (9, 23, 31, 34). These imbalances are thought to contribute to the onset and progression of the disease (11). The earliest changes of cartilage initiate from the joint surface. The surface is where the mechanical loads such as compression and shear are primarily borne (19). During the early stages of OA, the chondrocytes function to recuperate the degeneration of solid content by increasing its metabolic activity rate in an attempt to replace the degenerated collagen and proteoglycan (41, 42).

Normal articular cartilage health is maintained by compensatory responses of chondrocytes to mechanical loading including restoration of fluid and collagen content. However, excessive loading of articular cartilage beyond normal physiological values, will result in a net degeneration of the cartilage tissue as the chondrocytes start to decrease and cease their responsive action (3). This will lead the course of the disease towards late or severe stages where, for the most part, senescence of chondrocytes is observed, especially in articular areas closer to the surface. With longer-term progressive chondrocyte dysfunction, rigorous collagen degeneration, excessive mechanical loading, and remarkable molecular decomposition, the OA reaches its most severe stages (43).

1.2.5 Biomarkers in osteoarthritis

Upon degeneration and synthesis of articular cartilage, molecules responsible for these actions are released into the systemic circulation and have the potential to be used as evaluative tools for OA diagnosis and monitoring. In the case of OA, some of the common biomarkers are the ones with joint turnover regulatory roles (cytokines), collagen degenerating agents (matrix metalloproteinases - MMP), or cleaved parts of collagen molecules (C-terminal cross-linked type II collagen) (23). A number of studies have previously examined the role of cytokines and collagen particles in relation to OA, with considerably less attention on MMPs. As a result, the focus of this thesis was to examine the role of MMPs in knee OA pathology.

1.2.5.1 Matrix metalloproteinases

In 1962, MMPs were discovered by Jerome Gross and Charles M. LaPiere while studying the degeneration of triple-helical collagen during metamorphosis of a tadpole tail (44). MMPs are zinc and calcium-dependent endopeptidases, synthesized from inactive proMMPs and are then activated when cleaved by extracellular proteinases (45). MMPs consist of three domains: N-terminal propeptides, catalytic domain and Cterminal domain (46). MMP enzymes play an important role in the breakdown of ECM, also seen in normal physiological tissue remodelling processes like embryonic development and reproduction (47). A number of MMPs have been identified to-date, however only certain types of MMPs have been implicated in the breakdown of collagen. Some of these include MMP-1, MMP-3, and MMP-13.

1.2.5.1.1 MMP-3

The MMP-3 gene is a member of a cluster of MMP genes which localize to chromosome 11q22.3. The molecular weight of the MMP-3 enzyme is estimated to be 54 Kilodalton. This enzyme degenerates collagens type II, III, IV, IX, and X, proteoglycans, fibronectin, laminin, and elastin. MMP-3 can also activate other MMPs such as MMP-1, -7, and -9, making MMP-3 a crucial marker in connective tissue remodeling (45). Lohmander et al. showed that the baseline levels of MMP-3 measured in plasma can be a predictor of the knee joint space narrowing (JSN) in 120 women with unilateral knee OA over a period of 30 months (48). Specifically, they found that the baseline MMP-3 level was a significant predictor of JSN over the 30 month follow-up period. Another study reported that serum MMP-3 levels demonstrated an elevation in

people with mild and moderate stages of OA compared to people with severe OA (49). It was also shown that mRNA expression of MMP-3 in articular cartilage and synovial membrane samples from individuals with OA were higher than those of healthy individuals (50). In a recent study, Abd-Allah et al. reported a more frequent presentation of MMP-3 polymorphism in patients with OA and RA in comparison with healthy controls (51). Patients with knee effusion, as one of the characteristics of OA, showed higher MMP-3 levels in comparison to individuals without effusion in their knees (52). These results suggest that MMP-3 does have the potential of being counted as one promising biomarker for OA, though the relationship between factors that are directly related to the OA disease and the levels of MMP-3 needs to be noted and clarified.

1.2.5.1.2 MMP-1 and MMP-13

It has also been shown that MMP-1 and MMP-13 play important roles in cartilage degeneration by cleavage of type II collagen that is seen during osteoarthritic changes (53). MMP-1, or interstitial collagenase, breaks down a number of collagens such as collagen types I, II, III, VII, and (54, 55). Mitchell et al. showed that both MMP-1 and MMP-13 initially target the type II collagen at the same time; however, in continual processes of degradation, MMP-13 is responsible for further degeneration of collagen (53). It has been shown that the expression of MMP-1 is significantly higher in scaffolds implanted with osteoarthritic chondrocytes compared with healthy chondrocytes. Cells extracted from the cartilage near the areas with osteoarthritic changes showed expression of MMP-13 higher than those of normal chondrocyte (54). The

expression of MMP-13 mRNAs was also shown to be present in osteoarthritic chondrocytes, as well as the level of protein production that has been increased. Reboul et al. showed that in OA the MMP-13 protein released from chondrocytes is produced in higher levels than in the normal group (56). The results of a study by Billinghurst et al. support these findings. They reported that MMP-13 can influence the levels of collagen type II cleavage that is produced as a result of cartilage breakdown (57). It was also reported that the expression of MMP-13, along with a number of other cartilage degeneration biomarkers, are up-regulated in neighbouring areas of an arthritic cartilage lesion (58). Little et al. showed the important role of MMP-13 in cartilage changes. They demonstrated that MMP-13 deficiency in mice inhibits the erosion of cartilage and can prevent osteoarthritic changes (59).

1.3 Matrix Metalloproteinases and Load

Biomarkers may be a useful means of investigating OA; however, understanding their relationship with the pathogenesis of OA is of great importance. As indicated earlier, load is a known risk factor for degeneration of arthritic cartilage and thus, evidence of a relationship between loading and OA biomarker activity would be useful in determining the validity and utility of such biomarkers in the study of OA.

1.3.1 MMP-3 and load

A number of studies have tried to compare the physiological responses of articular cells in loaded and unloaded conditions. Comparing the effect of loading on different regions of the joint, in a mechanically loaded and a site-matched free-swelling control group, Bevill et al. showed that the levels of a number of cartilage molecules including Coll-II and MMP-3 were strongly up-regulated in the loaded areas compared to control sites (60). Lin et al. in an *in vitro* study investigated the effect of mechanical stress on the metabolism of chondrocytes overlying the subchondral bone. They co-cultured stressed and un-stressed osteoblasts with chondrocytes and observed a significant elevation in the expression of MMPs-1, 3 and 13 genes in the stressed group. They suggest that these results provide a possible explanation for the onset of OA in humans (61).

The magnitude of the applied load has been reported to be one of the contributing factors that lead to different levels of MMP-3 expression. In a recent study, Akamine et al. examined the affect of applying different magnitudes of load to a collagen scafold. They showed that MMP-3 can be released interstitially as a result of applying excessive magnitudes of cyclic load on synovial cells that were seeded onto a collagen scaffold (62). The study by Lin et al. revealed that high magnitudes of cyclic stress (15 KPa) can increase the expression of a number of MMPs, including MMP-3 (61). They suggest that changes in the metabolism of cartilage can be induced by stressing osteoblasts, including a possible explanation for the initiation and progression of OA. Injurious mechanical compression has also been reported to increase the levels of MMP-3 mRNA by 10-fold in the articular cartilage tissue explants from bovine femoropatellar groove within the first 24 hours after injurious mechanical stimulation (63). Finally, in a study conducted in 2008, Asundi et al. reported a difference between the MMP-3 expression in response to 4 megapascal (MPa) and 5 MPa of load in *in vitro*

conditions (64). The expression of MMP-3 was up-regulated up to 58 and 100 per cent for 4 and 5 MPa of load magnitude, respectively.

The manner in which the load is applied can differ, as well as the expressions of MMP-3. Nicodemus et al. showed that as a result of continuous loading of primary bovine chondrocytes encapsulated in polyethylene glycol hydrogels, the expression of MMP-3 was down-regulated by 2-fold, while the intermittent loading of the same cells led to an 8-fold up-regulation of MMP-3 (65). Therefore, intermittent loading, and not necessarily continuous loading, may play an important role in the regulation of MMP-3 levels. In another study, Zielinska et al. showed that dynamic compressive loading for two hours with a frequency of 1 Hertz (Hz) can up-regulate the expression of MMP-3 with a significant difference from the results of a non-dynamic loading group (66). These manifest the importance of the manner of the load application. For example, Muroi et al. applied a cyclic compression to synovium-derived cells that were cultured onto a collagen scaffold. They observed an up-regulation in MMP-3 mRNA and MMP-3 expression (67). Raïf et al. also exposed bovine synovial cells seeded onto an artificial scaffold to cyclic tensile strain and observed an 85% increase in MMP-3 activity under mechanical stimulus, in comparison to their control group which was not subjected to cyclic tensile strain (68).

The integrity of the source from which the cells are extracted is also a matter that has to be considered. Salter and Millward have reported that the chondrocytes, which were extracted from healthy individuals, interestingly showed a decrease in levels of MMP-3 in response to load, while the levels did not significantly change in osteoarthritic samples (69, 70). Another study in Massachusetts attempted to measure the up-

regulation of enzymatic degradative expression and cytokine-stimulated degradation in bovine cartilage. The extracted cartilage was exposed to injurious compression and, as a degenerative agent in cartilage turnover, the expression of MMP-3 mRNA was compared between two groups. Results revealed that the expression of MMP-3 mRNA had a 10-fold increase in the injuriously loaded cartilage group in comparison with the control group (63).

1.3.2 MMP-1, -13 and load

In an attempt to unveil the mechanisms of cartilage destruction caused by mechanical loads, one study in Japan showed that high magnitudes of cyclic loading can increase the mRNA levels of MMP-1 and MMP-3 (71). Another study in Germany looked at the differences in matrix turnover as a result of locating collagen cells to higher (2.5 MPa, 30 min, 0.1 Hz) and lower (0.25 MPa, 30 min, 0.1 Hz) hydrostatically pressurised environments. The extracted cells were seeded into three-dimensional collagen matrices and exposed to the different magnitudes of load. The results of this study showed that high hydrostatic pressure tends to increase the expression of MMPs-1, 3 and 13, while low hydrostatic pressure had no effect on the expression of MMPs-1, 2, 3 and 13 (72). Another showed that expression of MMP-1 and MMP-3 mRNAs is induced by exposing human synovial cells, seeded into a collagen scaffold, to excessive loading (62). Wenger et al. compared the effects of applying 10 and 30 atmospheres of loading on fibrochondrocytes, extracted from three, 2-week-old pigs, on molecular changes of the matrix and found that higher loads lead to more expression of MMP-1 (73).

The manner in which the load is applied onto the various components of cartilage is also important. Fehrenbacher et al. conducted an investigation into the comparison of molecular response in porcine cartilage explants to static and dynamic loading. They looked at a number of different ECM molecules including MMPs (MMPs-1, 3, 13, 14), tissue Inhibitor of metalloproteinases (TIMP) 1-4, aggrecan, and tenascin-c. Their results suggest that dynamic mechanical loading is capable of changing the MMP concentration within the articular cartilage with subsequent degeneration of collagen (74).

The integrity of the cells that undergo the application of load is also an important matter. For example, results of a study by Monfort showed a significant reduction in levels of MMP-1 expression in cells extracted from OA-free source. Put in the same environment, cells extracted from an osteoarthritic source did not manifest such decrease in MMP-1 levels.(55).

In summary, these studies indicate different instances of MMP protein and gene expression as a result of applying load to collagenous samples in *in vitro* and animal studies. Different aspects of load, including the magnitude of load and the manner of load application, were shown to be related to the manner in which MMP levels are expressed. However, due to ethical considerations, it is unfeasible to exert degenerative loads onto human articular joints for investigational purposes. Because of the lack of data regarding the relationship between the loading of the human joints and the expressions of MMPs, it cannot be indicated that the excessive amounts of load can necessarily influence the levels of MMPs in humans.

1.4 Measurement of Dynamic Knee Joint Load in Humans

Due to the unfeasibility of having a direct approach involving one's intra-articular knee joint space, measuring the amount of load that passes through the knee joint is problematic. An alternative method that is widely used for acquiring information about the quantities of intra-articular knee joint load is evaluating the knee adduction moment (KAM) obtained from three-dimensional motion analysis. Many studies have proposed that the KAM can be an indirect measure of the intra-articular knee joint load in the medial compartment during walking (75, 76). A substantial portion of total loading across the medial compartment of the knee joint is produced by the KAM, which is an external load that during gait forces the tibia into varus (75).



Figure 2. Depiction of the knee adduction moment.

KAM is the rotary force that tends to adduct the knee joint and is primarily calculated as the product of the ground reaction force (GRF) and the moment arm (denoted as the lever arm (LA) in this figure) of the ground reaction force [Source of figure (77)].

1.4.1 Measuring the knee adduction moment

The KAM is primarily calculated as the product of the resultant ground reaction force (GRF), which is the force exerted by the ground on a body in contact with it (78), in the frontal plane and the length of the frontal plane moment arm, which is the perpendicular distance between the GRF from the centre of rotation of the knee (79) (Figure 2). However, this definition has to be used with caution. In a motion analysis laboratory, the KAM is accurately calculated by considering every segment of the limb as a rigid body with a coordinate system designed to coincide with the anatomical axis (80).

1.4.1.1 Peak knee adduction moment

The characteristics of the KAM can be illustrated via a time-varying diagram during the stance phase of a walking gait (81). Peak KAM is the highest point of the KAM diagram that is usually seen at the peak of the first curve (Figure 3). The values obtained from peak KAM are indicative of the maximal load presented at one instance of the stance phase of gait. Several studies have shown that peak KAM is highly associated with different aspects of OA (82-84) and proposed it to be a promising measure for depicting the amounts of intra-articular knee joint load.

1.4.1.2 Knee adduction moment impulse

KAM impulse is the total area in the positive section of the KAM curve that takes into account the magnitude of load as well as the duration of stance (Figure 3). It has been shown that, in comparison with peak KAM, KAM impulse may be a more accurate measure for discriminating between mild and moderate OA severities (85, 86). KAM impulse has also been shown to have a more important relationship with the structural changes of OA than the peak KAM (81, 83).



Figure 3. Knee adduction moment impulse and peak knee adduction moment

Both the peak and impulse measures have been shown to be valuable measures for examining different aspects of KAM, although, considering their usefulness, it is ultimately the purpose of a given study that determines the application for each. For example, if one aims to evaluate the articular changes in response to a one-time vigorous loading of the joint, peak KAM can represent the maximal load that was applied to that joint; therefore, for this purpose it can be a better tool. On the other hand, if the purpose of a study is to demonstrate the cumulative effects of loading on osteoarthritic changes, like molecular changes in the serum, the KAM impulse appears to be a more appropriate measure since, by definition, it is the area under the KAM that indicates the total mechanical loading of the medial compartment of the knee during walking (85).

1.4.1.3 Knee adduction moment units

Given that it is a moment of force, the raw KAM values are reported in Newton meter (Nm) units, and KAM impulse, for including the element of time, is reported in Nm x second (Nm s) units. It is seen in many of the studies that comparison of KAM values are controlled for differences in height and weight (84, 87), where the values will be Nm/kg or % body weight (BW) x height (Ht) for peak KAM and Nm s/kg and % BW x Ht x s for KAM impulse (88). To the contrary , it has been mentioned that individuals with different body weights and heights do not show a significant difference in the tibiofemoral articulating surface of their knee joints; therefore, normalization of knee moments can sway attention from the actual amounts of intra-articular knee joint load (89, 90). To clarify, Robbins et al. conducted a study comparing diagnostic accuracy of normalized and non-normalized values of peak KAM and KAM impulse in knee OA. They showed that non-normalized values are significantly more accurate than the normalized values in distinguishing among OA severities and suggested that, for investigation of minute clinical changes, non-normalized values can be of more value
(88). Thus, for investigating the molecular level changes that are caused by load, nonnormalized values of KAM impulse appear to be the most appropriate measure.

1.4.2 Knee adduction moment and osteoarthritis

To demonstrate the usefulness of KAM as a valuable measure in OA studies, the relationship between KAM and different aspects of OA need to be described.

1.4.2.1 The relationship between knee adduction moment and osteoarthritis outcomes

The first study that looked at the KAM in people with knee OA was conducted by Prodromos et al. in 1985 (80). The purpose was to determine the relationship between knee joint loading during gait and the clinical outcome of high tibial osteotomy (HTO) surgery. The KAM was used as an indicator of the status of the knee joint loading in 21 participants that underwent an HTO (HTO group) as well as a control group consisting of 15 individuals, with the KAM of the HTO group measured before and after the surgery. After measuring the KAM of the HTO group, the participants were divided into two groups of high and low adduction moment groups according to the magnitude of the calculated KAM. Participants of these groups were similar in terms of preoperative knee score, initial varus deformity, immediate postoperative correction, age, and weight. After the HTO, the adduction moment group demonstrated a significantly lower average KAM after surgery in comparison to the high adduction moment group. It was also shown that, at an average 3.2-year follow-up, the clinical results of the surgery (pain, physical function,

and joint deformity) was considerably better in participants in the group with lower adduction moment at the preoperative measurements in comparison to those in the other group. It was also indicated that the group with the preoperative high adduction moment manifested a significant recurrence of varus deformity (80). This may be explained by a residual high knee joint load in the previously high knee adduction moment group. The results of Prodromos' study indicate that KAM can play an important role in determining the clinical outcome of studies that are designed to investigate the amounts of intra-articular knee joint load.

1.4.2.2 Knee adduction moment and intra-articular knee joint load

Despite the fact that KAM has been proposed to be an appropriate proxy for the amount of load that passes through the medial compartment of the knee joint, evidence that could demonstrate a direct link, *in vivo*, between the KAM and amounts of intraarticular knee joint load did not exist until Zhao et al. conducted an interesting experiment in 2007 (91). In this study, a load sensor was implanted inside the knee joint of one participant and data were simultaneously collected from the instrumented implant and laboratory-based motion analysis while the participant performed five different gait motions (normal, fast, slow, wide, and toe-out). The correlation between the KAM, obtained from motion analysis, and the total axial load that passed through the medial compartment of the knee joint, obtained from the knee implant, was investigated. The results revealed that KAM was significantly correlated with the axial load applied to the medial compartment of the knee joint (R^2 = 0.69, *p*>0.001). The findings of this study

suggest that a direct link exists between the KAM and the quantities of load that pass through the medial compartment of the knee joint.

1.4.2.3 Knee adduction moment and osteoarthritis progression

As mentioned before, load is one the main risk factors that contribute to the onset and progression of OA. Thus, the question to ask is whether or not the KAM values can be used as an indicator of the changes in the severity of knee OA. A study by Miyazaki et al. (82) holds the answer to this question. In an attempt to predict the radiographic disease progression via knee dynamic load at baseline, Miyazaki et al. collected data from motion analysis and radiography from 106 patients with OA in the medial compartment of their knees and compared these values after six years in 74 of the available participants. They used Altman's definition of radiographic disease progression, defined as a narrowing of the minimum joint space of the medial compartment for more than one grade (92). The results of this study revealed that the baseline KAM was higher in 32 of the patients who showed disease progression compared to those who did not manifest progression of OA. The amount of JSN over the period of six years had a significant correlation with the baseline KAM. They reported that risk of knee OA progression increases 6.46 times when the KAM increases 1% BW x ht. These results suggest that KAM can play a predictory role in determining the risk of OA progression in the medial compartment of the knee joint over a relatively long period. Another study that investigated the relationship between the KAM and the progression of OA was conducted by Bennell et al. (93). This longitudinal cohort study aimed to investigate the associations between KAM values and loss of

medial cartilage volume over 12 months in 144 patients with medial compartment knee OA. Biomechanical data, indicating the KAM peak and KAM impulse values, as well as baseline and follow-up magnetic resonance imaging (MRI) from the knee joints for assessment of the volume of the medial tibial cartilage plate, were studied. An association was observed between the KAM impulse and changes in cartilage volume in a way that higher KAM impulse at baseline was associated with greater medial tibial cartilage loss over 12 months. These findings suggest that high KAM values can be considered as a risk factor for disease progression.

1.4.2.4 Knee adduction moment and osteoarthritis severity

KAM has also been shown to be related to the severity of OA. Thorp et al. (85) investigated the differences of the knee joint loading patterns between participants with differing grades of radiographic OA based on the Kellgren and Lawrence (K/L) scale (94). They recruited 28 participants without knee OA (K/L 0 or 1) and 23 participants with symptomatic OA (K/L 2 or 3) and calculated their KAM using gait analysis. They looked at the KAM impulse and peak KAM over the whole stance and in the four subdivisions of stance. The results illustrated an increase in both peak KAM and KAM impulse with radiographic severity. However, while the KAM impulse was shown to be significantly different between those with grade 2 and grade 3 of K/L scale, no differences were found in the peak KAM value. In an earlier study, Sharma et al. (95) investigated the association between the KAM and the severity of knee OA after controlling for age, sex, and pain level. For these purposes, they recruited 54 patients with medial tibiofemoral OA and used gait analysis and radiographic imaging to

evaluate the grade of knee OA. It was shown that KAM correlated with the K/L grade in both knees, and also with joint space width in both knees. They reported that for a 1.0unit elevation of KAM (%BW x ht), a 0.63 mm of JSN is likely to occur. Based on these results, the authors suggest that there is a significant relationship between KAM and OA disease severity.

Higher KAM is indicative of greater amounts of load applied to the knee cartilage, while this excessive loading is the main contributing factor in the breakdown of knee cartilage. The provided information indicates that KAM can be used as a valid value for determining the magnitudes of load that pass through the knee joint. By having the possibility of measuring the amounts of intra-articular knee joint load, the opportunity for looking at the OA articular changes with respect to its risk factors, including the load, age and gender of individuals, becomes available.

1.5 Thesis Rationale and Objectives and Hypothesis

1.5.1 Thesis rationale

Despite the numerous advantages that MMPs can provide in OA studies, there are limitations that currently hold clinicians back from using them as definitive diagnostic tools. Many studies have shown significant relationships between MMP levels and different OA characteristics, as well as with different loading stimulations in animal and *in vitro* studies; however, to-date no solid evidence indicating a direct and meaningful relationship between the intra-articular knee joint load and MMP levels exists in humans. It is not clear how much of the variance in MMP levels is due to the effect of dynamic knee joint loading. Another matter would be the sensitivity of biomarker level changes to factors other than knee joint load, meaning the obtained values might be influenced by factors not attributable to OA. An inflammatory condition, like periodontitis for example, can cause an elevation in expression of MMPs-1 and 3 (96). The latter can considerably affect one's understanding of the OA-biomarker relationship; therefore, confounders ought to be detected and ruled out.

These limitations support the necessity of an effective strategy for validation of OA-specific biomarkers. For MMPs to be confidently used in clinics to assess and monitor patients with knee OA other risk factors must be able to determine an acceptable percentage of MMP level changes. Identification of positive outcomes in testing the validity of these biomarkers will provide sufficient reason for medical companies to invest in mass production of OA-specific diagnostic kits, which can be widely used in medical laboratories.

1.5.2 Thesis objectives

The primary objective of this thesis was to determine whether or not there is an association between the levels of MMPs and knee joint load, as measured by the KAM.

1.5.3 Thesis hypothesis

It was hypothesised that there would be a statistically significant association between MMP levels and KAM of the knee. Specifically, it was hypothesized that: a. There would be a statistically significant positive association between MMP-3 levels and KAM

b. There would be a statistically significant positive association between MMP-1 levels and KAM

c. There would be a statistically significant positive association between MMP-13 levels and KAM

It was also hypothesised that other OA-related factors can influence MMP levels.

- a. MMP levels can be influenced by the age of a person
- b. MMP levels can be influenced by the OA severity of a person

Chapter 2: Methods

For the purposes of this cross-sectional study, participants were recruited to be categorized as either having knee OA (OA group) or not (Control group). The participants in the OA group were recruited for a previous study (97), which had the same screening procedures as the current study. This procedure consisted of a phone screening phase and a radiographic screening phase (please see sections 2.1.1.1 and 2.1.1.2). After approving the eligibility of participants, they were invited to the UBC Hospital for blood sampling as well as demographic and biomechanical data collection. The analysis of biomechanical data took place at the Motion Analysis and Biofeedback Laboratory (MABLab) at the UBC Hospital, and the analyses of serum samples were performed at the Heritage Medical Research Building at the University of Alberta and at the Laboratory Medicine at Vancouver General Hospital (VGH). Multiple linear regression analysis was used to investigate the relationship between the independent variables including KAM, age, and severity of OA, and the dependent variables, the MMP levels.

2.1 Study Participants

People with knee OA were recruited from a previous study that aimed to investigate the effect of exercise on biomarker levels (97). Using a non-probability accidental sampling technique, participants were recruited from the community using advertisements in print media and underwent the same screening procedures (phone screening and radiographic screening) as described below in sections 2.1.1 and 2.1.2. Written consent indicating participants' permission to use their data and samples for further investigational purposes was collected.

For recruitment of healthy control participants, those who appeared to meet study inclusion/exclusion requirements were recruited via accidental sampling. Screening took place over two phases: initial phone screening for study eligibility and radiographic screening to rule out OA.

2.1.1 Initial phone screening for study eligibility

The purposes, procedures, and significance of the study were explicitly explained during a phone interview or in a face-to-face meeting with interested volunteers. The prospective participants were informed about their rights, and were informed of the fact that their consent was mandatory for inclusion into the study. As mentioned before, the population of this study consisted of individuals radiologically diagnosed with knee OA and healthy individuals (Control group). For each, certain criteria were defined:

2.1.1.1 Inclusion criteria

For <u>both</u> groups:

- 50 years of age or older

- Ability to speak, to read and understand English. The reason for this is that we would expect our study participants to be able to understand and follow our study rationales and instructions and also the information within the consent form.

For the <u>OA group</u>:

- OA in the medial compartment of the knee joint. (K/L grade 2 and 3).

- Knee pain during walking, greater than 3/10 (based on an 11-point scale; 0 =

"no pain", 10 = "maximal pain") on most days of the previous month.

For the healthy control group:

- No definitive OA in the medial or lateral compartments of the knee (K/L grade 0 or 1).

2.1.1.2 Exclusion criteria

For <u>both</u> groups:

- History of knee replacement surgery.
- Recent use of corticosteroids (oral or via injection).
- Arthroscopic knee surgery within the last six months.
- Inability to ambulate without a gait aid.

- Presence of inflammatory arthritic conditions, systematic joint diseases, or any type of inflammation.

For the OA group:

- OA in the lateral compartment of the knee more than the medial compartment.

- Grade 4 of K/L classification for OA. It has been shown that the levels of biomarkers specific for collagen degeneration decrease dramatically in serum samples of grade 4 K/L osteoarthritic patients (73). This can be explained by considering the difference in the histology of the cartilage and subchondral bone tissues which are under degradation in grade 4 OA. In these individuals, degeneration of Col II is related to the degeneration of subchondral bone; hence, tracing the biomarkers of collagen breakdown will not be as informative as it is in samples collected from those with lower grades of OA severity.

For the healthy control group:

- Any knee or lower limb joint pain.

2.1.2 Radiographic screening

Individuals who did not have recent (within six months) radiographs of their knees were referred to the False Creek Diagnostic Radiography Centre for standard xray acquisition. Dr. Hunt assessed all x-rays to determine each participant's eligibility based on radiographic inclusion criteria. Radiographs were evaluated for the presence or absence of OA using the K/L classification system (94). The K/L scale of OA classification (Table 1) was developed in 1957 and is still one of the most widely used scales for determining the stage or severity of OA. This scale classifies OA into four groups, each indicating particular signs in the radiography image of the target joint. The radiological features used for considering evidence of knee OA are: 1) osteophyte formation on the bone margins, and 2) the JSN between the femur and tibia. Grade 0 can be seen in healthy knee joints. Minute osteophytes can be observed in grade 1, though it still cannot be determined as certain OA. In grade 2, definite osteophytes are observed but without the JSN between the bones. When, along with osteophyte formation, the knee joint space is reduced, the knee can be given the grade 3 of K/L, and finally, grade 4 of K/L is indicative of excessive JSN and osteophyte formation. The degeneration at this grade takes place in the subchondral bone and essentially there is not much collagen left to be degenerated. Those who passed the x-ray screening were invited to UBC for a one-time data collection session.

| Grade of the disease | Description |
|-----------------------|---|
| Grade 0 (free of OA) | No osteophytes, no JSN |
| Grade 1 (no OA) | Possible osteophyte formation, doubtful JSN |
| Grade 2 (mild OA) | Definite osteophyte formation, doubtful JSN |
| Grade 3 (moderate OA) | Definite osteophyte formation, definite JSN |
| Grade 4 (severe OA) | Large osteophytes, marked JSN, definite deformity of bone contour |

Table 1. Kellgren and Lawrence grading scale

2.2 Data Collection

Participants reported to the UBC Hospital for serum sample and biomechanical data collection as well as to provide more clinical and demographic information.

2.2.1 Demographic and clinical information

Participants who passed the process of phone screening and met the further radiographic inclusion criteria were invited to the MABLab for collection of data including demographic and clinical data. The MABLab is on the 3rd floor of the UBC Hospital. Information about age, lower limb injury and surgery history, any used medication, their last meal, and tobacco and alcohol consumption were collected.

2.2.2 Serum sample collection

Participants were referred to the outpatient clinic in Laboratory Medicine, on the main floor of the UBC Hospital, to have blood samples collected. Samples were labelled with a unique identifier and date, and then stored. Blood was centrifuged after clot formation, and serum was aliquoted into 1.8 ml aliquots and stored at -80 degrees Centigrade. Tracking to link specimen ID to participant ID was maintained in a study database, available only to Dr. Hunt. After all samples were collected, they were sent to Dr. Ghahary's laboratory for storage and then to Dr. Maksymowych's laboratory and VGH Laboratory Medicine for analysis.

2.2.3 Biomechanical data

Participants underwent gait analysis for assessment of the dynamic loading of the knees. Using the Helen Hayes marker set (98), 22 reflective spheres were placed on major landmarks on the participants' bodies including the posterior aspect of calcanei, lateral ankle malleoli and knee epicondyles, middle of the tibial shafts and thighs, the anterior superior iliac spines, sacrum, the olecranons, posterior wrists, acromions, and finally, one sphere was randomly placed on the right scapula. The latter enables the computer programme not to confuse the sides. Participants were filmed by 8-reflection-sensitive cameras (Hawk; Motion Analysis Corporation, Santa Rosa, CA) at the sampling rate of 120 Hz, connected to Cortex, which is the software for the motion analysis system. For collecting static data, needed as a reference point for detecting changes in the spatial displacement of body segments, four extra reflective spheres were placed on the medial malleoli and the medial knee joint lines. Our participants were asked to maintain a standing anatomical position for about 10 seconds, while standing within the edges of one of the two floor-mounted force platforms (Advanced Mechanical Technology Inc., Watertown, MA) implanted in middle of a 10 meter walkway, collecting GRF data at the sampling rate of 1200 Hz. This walkway is surrounded by the aforementioned cameras that are capable of sensing any movements made by the participants by detecting displacements of the reflective spheres in space. Force platforms were also connected to Cortex and collected external forces exerted on the participants as they stepped onto the platforms. The data obtained from the platforms were synchronized with the data obtained from the cameras.

Participants were asked to walk back and forth at their natural pace,

approximately ten times along the pathway. The goal was to have the participants hit the platforms without their feet touching the edges of the platform while walking at their natural pace. The reason for this consideration was that the characteristics of the GRF, recorded from trials in which a step has exactly landed within the edges of the force platform square, can be representative of the actual GRF values. Trials with such an attribute were referred to as good trials, and five of the good trials for each foot were randomly selected for further analysis. The trials in which the feet touched the edges of the platform were not taken into account.

2.3 Data Analysis

2.3.1 Serum sample analysis

Levels of MMPs were measured in Dr. Maksymowych's laboratory at the University of Alberta, via an analytical technique called enzyme-linked immunosorbent assay (ELISA). An ELISA test uses chemicals and components of the immune system to detect immune responses in the body (for example, proteins like MMPs). There are different types of the ELISA test, but the most basic type consists of an antibody attached to a solid surface. This antibody has the affinity for the substance of interest. For example a mixture of purified MMP-3 linked to an enzyme and the test sample (blood, urine, etc.) is loaded into the test system. If no MMP-3 is present in the test sample, then only MMP-3 with a linked enzyme will bind. The more MMP-3 available in the test sample, the fewer enzymes linked to MMP-3 will bind. The substance that the enzyme acts upon is then added, and the amount of product is then measured by using methods like measurements of the colour changes in the compound. For investigating the colour changes an ELISA-Reader can be used, the optical density can be observed, and the final outcome is recorded in ng/ml units. ELISA is generally accepted to be a very accurate method of measurement (99).

For our analyses, we used Biorad's human MMP kits (Human MMP-1, Human MMP-2, Human MMP-3) with the sensitivities of 8 pg/ml, 0.3 ng/ml, and 6 pg/ml, respectively. All samples were tested in duplicate and the average values were computed for each sample. It was found that the coefficient of variation was less than 10% for each kit.

Levels of serum C-reactive protein (CRP), as a generic indicator of inflammation, were measured in the Laboratory Medicine at VGH. CRP is an acute-phase protein that shows a serum level elevation during general and non-specific responses to infectious and non-infectious inflammatory processes and serves as a biomarker for systemic inflammation (100). CRP is synthesized in the liver and can be traced in serum or plasma (101). Elevation in CRP values is associated with pathological conditions; therefore, the CRP measurement can provide useful information for diagnosis, therapy, and monitoring of inflammatory processes. Polystyrene particles coated with monoclonal antibodies specific to human CRP were accumulated when mixed with CRP-containing samples. These accumulations scatter a beam light passed through the sample. The intensity of the scattered light is proportional to the concentration of the available CRP in the sample. The attained results were then compared with the standards of known concentration. MMP and KAM data from participants with CRP

levels above 4.0 mg/L were re-examined post-hoc to determine if they were within normal ranges. Participants with excessive CRP and MMP or load values were excluded from analysis.

2.3.2 Biomechanical data analysis

The post-processing of gait data was conducted using commercially available software (OrthoTrak; Motion Analysis Corporation, Santa Rosa, CA). Based on inverse dynamics principles, the obtained data were used to compute external moments about the knee joint. In biomechanics, inverse rigid-body dynamics is used to calculate the moments of forces based on the kinematics of a body and that body's inertial properties (102). The thigh, shank and foot segments were modeled as a rigid body with a local coordinate system that coincided with anatomically relevant axes. The inertial properties of lower limb segments were anthropometrically approximated, and with respect to neutral positions that were defined during the static trials, translations and rotations of the mentioned segments were reported.

To quantify the KAM impulse values for each knee, the area under the KAM curves obtained from acceptable walking trials were calculated. Average values were caculated by averaging across the five trials for each participant. The KAM impulse values were reported in Nm.s units.

2.4 Sample Size Calculation

This study aimed to look at the way that a number of explanatory (independent) variables could determine the amounts of a dependent factor (MMP levels in serum), and the effect was decided by the degree of this association. No preliminary data were available, so a literature search was performed for previous research or pilot data, containing information directly related to this study. This study contained an entirely novel approach to the understanding of knee OA by bringing together the measurement of MMPs and a number of its explanatory factors such as KAM, age, and severity of OA.

Medline (Ovid SP) was searched for the MeSH term osteoarthritis and the results were "Or"ed with results from a keyword search for knee adduction moment. The MeSH term for matrix metalloproteinase was conducted and the results were "Or"ed with a keyword search for MMPs. A MeSH term and key word search were also done and "Or"ed for age and osteoarthritis severity. The "Or"ed results were then "And"ed to find the studies commonly looking at KAM, age, OA severity and MMPs in osteoarthritis. No previous studies have taken into consideration all of these factors together. Therefore, we had to use the standard power and effect size for determining the sample size for this multiple regression study. Considering an anticipated R² value of 0.50, a statistical power level equal to 0.80 and a p-value of 0.05 for a multiple regression with three predictors, the minimum number of participants required for this study was 26. To account for potential loss of data, 28 participants were recruited. The statistical calculations were conducted using the Statistics Calculators version 3.0 online at www.danielsoper.com/statcalc3/calc.aspx?id=1#. To account for potential loss of data,

or untraceable values, during MMP analysis we aimed to recruit 28 individuals to this study.

2.5 Statistical Analysis

To investigate the relationship between independent and dependent variables, multiple linear regression analysis was used. Multiple linear regression analysis attempts to fit a regression line for a response variable using more than one explanatory variable. The dependent variable in this study was the level of MMPs that were measured. Specifically, we measured the levels of MMP-1, MMP-3, and MMP-13 in all samples. The explanatory variables were those with a predictory role in determining the levels of MMPs and they included KAM, OA severity, and age. Using multiple linear regression, we evaluated the amount of variance in MMP levels explained by KAM, severity of OA and age of the participants.

For the KAM, we considered the KAM impulse (KAM imp) which reports the magnitude of load applied to the knee as well as the duration of stance phase in one stride. Also due to the fact that the biomarkers are expected to be released in response to loading applied to both knees, the amounts from summation of the KAM impulse values from the right and left knees (KAM imp Total) were calculated and used in the regression model. For the same reason, the severity of OA was reported as the sum of the K/L grades from right and left knees (K/L Total).

The order in which the variables were introduced to the regression models was determined based on their relationship with the MMP values. This was accomplished by

evaluating the association between each explanatory variable and the dependent (MMP) variable. Explanatory variables were then placed sequentially into the regression model starting with the variable with the smallest correlation with the dependent variable, followed by the second lowest, then finally by the most highly correlated explanatory variable. Analysis was performed using the Statistical Package for the Social Sciences (SPSS Inc, v. 21, Chicago. IL). Alpha was set to 0.05 for all analyses.

Chapter 3: Results

3.1 Participant Demographic and Clinical Outcomes

Serum samples were available from 17 participants with knee OA from the previous study and 18 serum samples were collected more recently from the participants of the control group. Thus, a total of 35 serum samples were sent to VGH Laboratory Medicine and University of Alberta for CRP and MMP analysis, respectively. One sample from the OA group and one from the control group with CRP levels above the predetermined score of 4.0 mg/L were excluded from any further analysis. This brought the number of samples down to 33, and after gender and age matching of the remaining participants, the final sample of 28 was attained. The number of males and females in both groups were equal (7 males and females in each group) and the average age was 63.5 years for both groups. The BMI average was 26.9 and 25.5 kg/m² for OA and control groups, respectively (Table 2). Of the 56 knees assessed for radiographic severity, seventeen knees were found to be completely free of OA (grade 0 K/L) while 12 showed to have possible osteophyte formation and doubtful JSN, yet no OA (grade 1 K/L). Nineteen knees had signs of mild OA (grade 2 K/L) and 7 knees revealed signs of moderate OA (grade 3 K/L).

| Subject Code | Sev | Age | Height | Mass | BMI | K/L | K/L | K/L |
|------------------|------------------|---------|--------|-------|---------|--------|--------|--------|
| Subject Code | Sex | (years) | (cm) | (kg) | (kg/m²) | Right | Left | Total |
| | Malo | 63.8 | 179.0 | 104.0 | 32.5 | 2 | 2 | 1 |
| KOABMKO2 | Malo | 60.5 | 173.0 | 114.0 | 36.0 | 2 | 2 | 4 |
| | Malo | 59.5 | 160.0 | 72.4 | 25.4 | 2 | 2 | 4 |
| KOABMKOA | Fomolo | 50.0 | 167.0 | 72.4 | 25.4 | 2 | 2 | 4 |
| | Molo | 70.0 | 107.0 | 74.4 | 27.7 | 3 0 | 3 2 | 6 |
| KOADMKOS | Male | 79.9 | 1/7.0 | 74.4 | 23.0 | 2 | 3 | C 4 |
| | Male | 50.8 | 109.5 | (1.5 | 24.9 | 2 | 2 | 4 |
| | Iviale Famala | 63.3 | 1/4.0 | 07.9 | 22.4 | 2 | 0 | 2 |
| | Female | 57.9 | 162.0 | /8.0 | 29.7 | 3 | 3 | 6 |
| | Female | 77.0 | 154.0 | 81.9 | 34.6 | 3 | 3 | 6 |
| KOABMIKTU | Male | //.0 | 161.5 | 61.0 | 23.4 | 2 | 2 | 4 |
| | Female | 63.1 | 151.0 | 64.0 | 28.1 | 2 | 2 | 4 |
| | Female | 56.8 | 1/2.0 | 67.8 | 22.9 | 2 | 2 | 4 |
| KOABMK13 | Female | 68.2 | 165.0 | 63.0 | 23.2 | 3 | 3 | 6 |
| KOABMK14 | Female | 66.3 | 152.0 | 52.0 | 22.5 | 2 | 2 | 4 |
| | | | | | | | | |
| Mean for OA | | 63.5 | 166.5 | 75.0 | 26.9 | | | |
| SD for OA | | 8.6 | 9.4 | 16.5 | 4.7 | | | |
| | | | | | | | | |
| CONBMK01 | Male | 59.0 | 174.5 | 66.4 | 21.8 | 0 | 0 | 0 |
| CONBMK02 | Female | 64.9 | 163.0 | 69.0 | 25.8 | 0 | 0 | 0 |
| CONBMK03 | Male | 78.2 | 171.0 | 71.7 | 24.5 | 1 | 1 | 2 |
| CONBMK04 | Female | 51.4 | 159.5 | 49.9 | 19.6 | 0 | 0 | 0 |
| CONBMK05 | Female | 73.9 | 151.7 | 65.6 | 28.5 | 0 | 0 | 0 |
| CONBMK06 | Female | 56.3 | 162.1 | 85.3 | 32.5 | 0 | 0 | 0 |
| CONBMK07 | Male | 53.2 | 177.0 | 69.9 | 22.3 | 1 | 0 | 1 |
| CONBMK08 | Female | 55.8 | 152.5 | 54.6 | 23.5 | 1 | 0 | 1 |
| CONBMK09 | Male | 61.1 | 173.0 | 62.6 | 20.9 | 1 | 1 | 2 |
| CONBMK10 | Male | 66.9 | 173.5 | 85.2 | 28.3 | 1 | 1 | 2 |
| CONBMK11 | Female | 66.6 | 155.0 | 64.8 | 27.0 | 0 | 0 | 0 |
| CONBMK12 | Female | 58.5 | 160.0 | 68.9 | 26.6 | 1 | 1 | 2 |
| CONBMK13 | Male | 72.3 | 166.4 | 68.0 | 24.6 | 0 | 0 | 0 |
| CONBMK14 | Male | 71.4 | 182.0 | 100.8 | 30.4 | 1 | 1 | 2 |
| | | | | | | | | |
| Mean for Control | | 63.5 | 165.9 | 70.2 | 25.5 | | | |
| SD for Control | | 8.3 | 9.5 | 12.9 | 3.7 | | | |
| | | | | | | | | |
| Mean for all | | 63.5 | 166.2 | 72.6 | 26.2 | | | |
| SD for all | | 8.3 | 9.3 | 14.7 | 4.2 | | | |
| SD for all | | 8.3 | 9.3 | 14.7 | 4.2 | | | |

Table 2. Demographic information

Abbreviations: BMI (Body mass index), K/L (Kellgren-Lawrence), KOABMK (knee osteoarthritis biomarker), CONBMK (control biomarker), SD (standard deviation)

The mean (SD) of KAM impulses for 28 right knees (KAM imp Right) was 13.1 (6.7) Nm.s (Table 3). The KAM imp left in 28 knees was 14.5 (8.1) Nm.s. No statistical significance was seen between the KAM impulses of the right and the left knees (p= 0.62). The mean (SD) value for KAM imp Total was 27.5 (14.3) Nm.s.

The MMP levels were reported in picograms per millilitre (pg/ml) for MMPs-1, 13 and nanograms per millilitre (ng/ml) for MMP-3. The mean (SD) of 28 MMP-1 values was found to be 9700.8 (5941.4) pg/ml and for MMP-3 it was 54.2 (26.2) ng/ml. It is important to note that only five samples revealed results after analysis of 28 samples for measurement of MMP-13. The mean (SD) of these five values was 201.2 (169.3) pg/ml. The absence of MMP-13 results in 23 of the 28 samples can be indicative of MMP-13 levels lower than the amounts that could be traced and reported via the implemented assay, which had the sensitivity of less than 6 pg/ml. For the purpose of extracurricular comparison of the aforementioned values between the OA and control groups, Tables 3 and 4 summarise the values for each group separately as well as providing summary data for the entire group of 28 participants.

| Subject Code | KAM imp | KAM imp | KAM imp | MMP-1 | MMP-3 | MMP-13 |
|------------------|--------------|-------------|--------------|---------|---------|---------|
| | Right (Nm.s) | Left (Nm.s) | Total (Nm.s) | (pg/ml) | (ng/ml) | (pg/ml) |
| KOABMK01 | 10.0 | 16.7 | 26.7 | 14645.0 | 44.1 | |
| KOABMK02 | 34.9 | 39.9 | 74.8 | 22793.4 | 51.4 | |
| KOABMK03 | 10.6 | 12.3 | 22.8 | 6346.8 | 62.8 | |
| KOABMK04 | 11.3 | 9.6 | 20.9 | 10465.2 | 25.8 | |
| KOABMK05 | 15.7 | 19.9 | 35.6 | 13921.0 | 85.8 | 98.3 |
| KOABMK06 | 10.9 | 12.7 | 23.6 | 6926.4 | 54.4 | |
| KOABMK07 | 12.6 | 12.3 | 24.8 | 16402.9 | 78.4 | |
| KOABMK08 | 17.7 | 12.2 | 26.9 | 5691.0 | 36.5 | |
| KOABMK09 | 27.6 | 29.4 | 57.1 | 9087.0 | 34.9 | |
| KOABMK10 | 16.7 | 16.4 | 33.0 | | 77.1 | |
| KOABMK11 | 12.4 | 11.4 | 23.8 | 11005.5 | 58.4 | |
| KOABMK12 | 8.5 | 6.8 | 15.2 | | 43.7 | |
| KOABMK13 | 5.6 | 16.0 | 21.6 | 8697.0 | 45.5 | |
| KOABMK14 | 7.4 | 8.1 | 15.5 | | 33.7 | 132.1 |
| | | | | | | |
| Mean for OA | 14.2 | 16.0 | 30.2 | 11452.8 | 52.32 | 115.2 |
| SD for OA | 8.0 | 8.9 | 16.5 | 5133.4 | 18.3 | 23.91 |
| | | | | | | |
| CONBMK01 | 10.0 | 9.1 | 19.1 | 3921.5 | 37.6 | 437.8 |
| CONBMK02 | 7.9 | 9.2 | 17.1 | 1862.7 | 21.8 | |
| CONBMK03 | 11.4 | 13.3 | 24.7 | 156.6 | 39.8 | |
| CONBMK04 | 7.2 | 3.0 | 10.3 | 181.5 | 33.7 | |
| CONBMK05 | 5.2 | 5.9 | 11.0 | 12967.7 | 34.6 | |
| CONBMK06 | 13.1 | 11.7 | 24.8 | 15357.8 | 31.7 | |
| CONBMK07 | 9.3 | 17.2 | 26.5 | 13174.5 | 67.8 | 312.3 |
| CONBMK08 | 5.4 | 7.7 | 13.0 | 11962.8 | 40.8 | |
| CONBMK09 | 19.8 | 29.4 | 49.2 | 1954.21 | 136.4 | |
| CONBMK10 | 19.4 | 19.8 | 39.1 | 12774.0 | 99.7 | |
| CONBMK11 | 10.3 | 12.5 | 22.8 | 16489.0 | 58.2 | |
| CONBMK12 | 12.4 | 5.1 | 17.5 | 3812.5 | 27.4 | |
| CONBMK13 | 19.2 | 20.7 | 39.9 | 5478.5 | 65.3 | |
| CONBMK14 | 14.5 | 17.7 | 32.2 | 16445.8 | 90.1 | 25.3 |
| | | | | | | |
| Mean for Control | 11.8 | 13.0 | 24.8 | 8324.2 | 56.1 | 258.5 |
| SD for Control | 4.9 | 7.3 | 11.7 | 6345.2 | 33.0 | 211.5 |
| | | | | | | |
| Mean for all | 13.1 | 14.5 | 27.5 | 9700.8 | 54.2 | 201.2 |
| SD for all | 6.7 | 8.1 | 14.3 | 5941.4 | 26.2 | 169.3 |

Table 3. Outcome measures

Abbreviations: KAM imp (knee adduction moment impulse), Nm.s (Newton meter second), MMP (matrix metalloproteinase), pg/ml (picogram per millilitre), ng/ml (nanogram per millilittre)

3.2 Multiple Regression Models

Based on bivariate correlation analysis, no explanatory variables were significantly correlated to MMP-1. Specifically, neither K/L total (R=0.119, p=0.573), age (R=0.20, p=0.339), or KAM imp (R=0.348, p=0.089) were found to be significantly related to MMP-1. The same was found for MMP-13 (p>0.12). In contrast, the KAM imp was significantly correlated with MMP-3 (R=0.44, p=0.02).

Bivariate correlational analyses was also used to determine the order of placing the variables inside the model and K/L grade was the first explanatory variable input into each regression model. K/L was able to explain 1.4%, 0.0%, and 53.9% of the variance in levels of MMPs-1, 3, and 13, respectively. However, none of these models were found to be statistically significant (p>0.16). The next variable entered into the model was age and our findings showed that age together with K/L grade could explain 5.5%, 8.2%, and 63.7% of the variance in MMPs 1, 3, 13, respectively. No statistical significance was seen for either of these findings (p>0.14). Finally, with KAM imp Total, the three variables together were able to explain 13.1%, 23.9%, and 65.8% of the variance in MMP-13, respectively. Only for MMP-3 was this found to be significant (p=0.036). While controlling for K/L Total and age, KAM imp Total was able to explain 7.6%, 15.7% and 2.1% in additional variance in MMP-1, MMP-3, and MMP-13, respectively.

| Table 4. Summary of | regression | models fo | r predicting | the matrix | metalloproteinases | levels |
|---------------------|------------|-----------|--------------|------------|--------------------|--------|
| , | 0 | | | | | |

| Model | R | R ² | R ² change | Р |
|--------------------------|-------|----------------|-----------------------|-------|
| (a) MMP-1 | | | | |
| K/L total | 0.119 | 0.014 | 0.014 | 0.573 |
| K/L Total + Age | 0.234 | 0.055 | 0.041 | 0.538 |
| K/L total + Age + KAMimp | 0.362 | 0.131 | 0.076 | 0.390 |
| (b) MMP-3 | | | | |
| K/L total | 0.016 | 0.000 | 0.000 | 0.934 |
| K/L Total + Age | 0.287 | 0.082 | 0.082 | 0.148 |
| K/L total + Age + KAMimp | 0.489 | 0.239 | 0.157 | 0.036 |
| (c) MMP-13 | | | | |
| K/L total | 0.734 | 0.539 | 0.539 | 0.158 |
| K/L Total + Age | 0.798 | 0.637 | 0.098 | 0.539 |
| K/L total + Age + KAMimp | 0.811 | 0.658 | 0.021 | 0.846 |

Abbreviations: R (coefficient of correlation), R^2 (coefficient of determination), R^2 change (change in the coefficient of determination), *P* (*P*-value or calculated probability)

The bivariate relationship between KAM imp Total and levels of MMPs- 1, 3, 13 are plotted for all participants in Figures 4 a, b, c. MMPs- 1 and 3 demonstrate a positive linear correlation, so that an increase in KAM imp Total elevates the MMP levels (Figure 4 a, b). The scatter plot created for showing the relationship between the KAM imp Total and available MMP-13 values (n=5) shows a regression line with downwards inclination (Figure 4 c), indicating a negative relationship between these two variables based on our limited number of available samples.









Scatter plots illustrating the relationships between total knee adduction moment and matrix metalloproteinase levels in serum samples collected from a study group consisted of OA and control participants.

Chapter 4: Discussion

This study provides new information about the characteristics of degenerative biomarkers of articular cartilage relevant to the study and prevention of knee OA. Specifically, data from this study details information regarding the relationship between degenerating cartilage enzymes or MMPs and KAM a measure of dynamic knee joint load – a potential mechanism of OA biomarker production — while controlling for other contributing factors such as age of participants and severity of knee OA. Findings of this study can improve our understanding of OA and may be useful for clinicians for preventive, diagnostic, and monitoring purposes.

4.1 Interpretation of the Findings

Many studies considered biomechanical and biomedical aspects of OA separately, though few have attempted to address the explanatory role of dynamic knee joint load in the variance of cartilage degenerating molecule levels. To our knowledge this is the first study that brings together three OA-related factors for the purpose of predicting MMP molecular changes, as well as for determining the role of KAM solely in explaining the levels of MMPs. According to the findings of previous studies, MMPs are one of the most important groups of molecules with a significant role in articular cartilage degeneration; therefore their levels may be viewed as biomarkers of OA (48,

49, 103). Dynamic knee joint loading, age, and OA severity were three factors that were taken into account as OA-related factors in the current study. The sum of KAM impulse from both knees of 28 participants along with their age and severity of OA could explain 24 per cent of the variance in MMP-3 levels. These findings suggest that MMP-3 can be viewed as a possible biomarker for OA diagnosis and progression, and results from measurements of its levels can be cautiously used clinically and for research purposes for investigating degenerative changes of articular cartilage in the knee.

The first factor that was added in the regression models was OA severity. Adding the K/L total scores in the regression equations for MMPs-1, 3, and 13 revealed the R² values of 0.014, 0.0, and 0.539, respectively, though none of these were statistically significant. These findings can be explained in different ways. One possibility is the unsuitability of MMPs1, 3, 13 in knee OA studies, although this interpretation should be made with caution. Previous studies, with larger sample sizes, have shown that MMP levels can alter significantly between different K/L grades (49), meaning that both MMPs and K/L grading scale are a valid means of investigation. Another interpretation that could be made is that for a delicate procedure like measurement of biomarkers produced as a result of cartilage breakdown, and minimally released into the blood stream, a five grade scale may not be most efficient tool for demonstrating the relationship between these changes and the severity of knee OA. These interpretations suggest that it is unlikely to find a direct relationship between OA severity graded by K/L and molecular changes of articular cartilage in the knee in small samples, also that the K/L grading cannot be looked at as the sole determinant of MMP changes.

The age of participants was another potential explanatory variable that was added to the regression model in the current study. The importance of age in OA has been stipulated repeatedly in the literature. The prevalence of OA is higher in elderly and symptomatic osteoarthritic changes appeared mostly after age of 50 (8, 10, 104, 105). It has also been shown that a positive correlation exists between different MMP levels and the age of the donors of the samples in which the MMP levels have been investigated (106, 107). The correlation between age and MMPs-1, 3, 13 revealed R² values of 0.04, 0.082, 0.6, though none were found to be statistically significant (p= 0.339, 0.140, 0.124 respectively). These findings suggest that, despite of the possible role that age can play in the development of OA and expression of cartilage degeneration, it cannot necessarily be looked at as an independent factor to explain the MMP variances. This finding can provide an explanation for the commonly asked question that 'if age a risk factor for development of OA, why is it that not every individual over the age of 50 has OA?' A possible answer to this question is that even though age plays an important role in development of OA, it cannot be looked at as a factor that solely causes OA.

Excessive loading is one of the main risk factors in OA development and progression and it was shown to be related to expression of MMP-3 in the current study. A number of studies, including animal and *in vitro* studies, have shown that application of high magnitudes of load leads to expression of molecules, in the same manner as can be seen in OA. Radin et al. have shown that as a result of applying excessive loads to a knee joint, dissected from a cow, the molecular expression that was seen in the experimental buffer was similar to those of osteoarthritis changes (21). They also

examined the effect of repetitive loading on knee joints of rabbits and showed that the changes that were caused by the excessive loading were consistent with those of degenerative joint disease (20). The results of the current study support the findings of Radin's and colleagues in terms of the molecular changes that take place inside the knee joint in response to load. A significant association was seen between KAM imp and MMP-3 variance (R^2 =0.19).

In vitro studies also have identified ways in which the MMP-3 gene and protein can be expressed in response to different aspects of load application. Excessive magnitudes of cyclic compressive load were applied to human synovial cells, in threedimensional cultured tissue, and a MMP-3 high expression was observed (62). It is important to note that physiological loading is an essential requirement for preserving joint integrity (108). Adequate amounts of load guarantees the balanced expression of MMPs and their inhibitors (TIMPs) in a healthy joint, while this harmony is disturbed in the absence of physiological loading (109). On the other hand, when the applied load exceeds a certain amount MMP expression dominates the inhibitory effect of TIMPs and matrix degeneration will occur (110). This is in line with the results of the current study that revealed a positive correlation between dynamic knee joint load and MMP-3 levels, in a way that elevation in KAM imp values could increase the upward inclination of the regression line between KAM Imp and MMP levels (Fig 4 a, b).

Another important matter that must be considered is the manner of load application. The mechanotransductive responses of chondrocytes can vary due to the frequency of the applied load. It has been shown that cyclic loading of the cartilage cells, seeded inside collagen scaffolds, can vary significantly from the response of

statically loaded groups (68). These findings can elucidate the reason for expression of MMPs in knee joints that were exposed to excessive and repetitive loading, as in ambulation an individual needs to shift his/her body weight on both knees. Due to different factors such as body asymmetry and unequal distribution of load between two knees, one knee can be susceptible to developing osteoarthritic changes more than the other one. For these reasons, the chances will be even higher when over-weight and unequal load distribution are accompanied.

In OA, a combination of risk factors determines the variations in molecular expressions. Age, dynamic knee joint loading, and OA severity were selected as the explanatory variables of this study. To the best of our knowledge, this was the first study that looked at the relationship between OA risk factors, most importantly dynamic knee joint loading, and MMP expressions in a human population. Together, the explanatory variables explained 24% of the variance in MMP-3 levels. This suggests that MMP-3 may be used as a promising biomarker in OA investigations. Different studies have identified MMP-3 as one of the most important collagenase enzymes in connective tissue remodelling, not only as a collagen degenerator, but also because of the regulatory role that MMP-3 plays in activating other MMPs (45). The MMP-3 variance can be expected to be more highly associated with the factors that are related to the degeneration of the cartilage or OA-related factors, dissimilar to what we found for MMPs-1 and 13.

The absence of significance in the association between MMP-1 and 13 with knee joint load can be due to a number of different factors. First, these biomarkers may not be the best indicators of osteoarthritic changes. However, previous studies have shown

that both these MMPs play significant roles in OA (53). It has been shown that MMP levels can be influenced by various factors, which are not necessarily associated with OA. Inflammation, for example, is one factor that can influence MMP levels (96). In an attempt to control the effect of inflammation, we excluded samples with higher than standard CRP levels; however other unknown factors influence MMP levels. Future studies should aim to detect new confounders and consider their role in OA studies.

An important issue that must be considered is that the presentation of biomarkers is controlled and balanced by their inducers and inhibitors. As mentioned before, TIMPs have an inhibitory role in production of MMPs (74), so if one desires to have a detailed understanding of MMP levels in serum, he/she must also look at the TIMP levels along with the MMPs. This can provide a possible explanation for our ability to detect MMP-13 levels in only five samples. MMP-13 is one MMP that can be down-regulated by all types of TIMP (111).Therefore, future studies should aim to analyse TIMP levels in combination with MMP levels when determining the role of MMPs in OA.

4.2 Study Limitations

Despite the new information that this study adds to the literature, the interpretation of the findings is limited. The level of MMPs that were examined was obtained from the serum of the participants, while biomarkers produced during the process of joint degeneration are initially released into the knee joint cavity containing synovial fluid (112). As a result of the effusion, some of these biomarkers, which are initially available inside the synovial fluid, are transferred into the circulation, meaning

that the amounts being measured inside the blood serum are not necessarily direct indicators of the degeneration process. Some investigators who have accessed the synovial fluid of the patients have examined the biomarker levels directly from the initial source (112, 113). Due to the invasive nature of this approach we chose not to collect synovial fluid samples. It is also important to note that different physiological conditions can possibly affect the levels of OA biomarkers. Even though we attempted to eliminate the effect of confounding factors, there is still a possibility for other unknown factors, including bodily or environmental factors, to influence the presentation of the biomarkers.

To our knowledge, we used two important outcome measures (MMPs and KAM impulse) for demonstrating molecular changes due to OA and dynamic knee joint loading. Several studies provided evidence for using both of these measures (51, 95); however, they should be taken into account with caution. In this study, only three members of the MMP family (MMPs-1, 3, 13) were decided to be looked at as biomarkers for OA. This decision was based on an extensive literature search and a number of consultations with experts in the field. Other MMPs may play an important role in OA and be related to dynamic knee joint loading, but were beyond the scope of this study.

It is important to reemphasize that MMPs are not the only molecules that could be of benefit in study of OA. Different researchers have looked at a number of different biomarkers for understanding OA (114, 115); nonetheless another reason that drew our attention towards MMPs was the matter of feasibility. For attaining a better understanding of the molecular changes, investigation of the cytokines that up-regulate

the target MMPs, as well as TIMPs which inhibit the production of MMPs, could have been highly useful. Due to the shortage of time and resources it was not a possibility for us to undertake an encompassing study which looks into the levels of the aforementioned molecules. Future research can benefit from additional analyses of these other biomarkers that may be noteworthy in the study of OA and may also explain MMP values measured in serum or synovial fluid.

KAM is a well-accepted measure of the load in the medial compartment of the knee joint (91), and it has been shown to be of great validity in OA studies (82, 85, 116, 117). However, KAM does not necessarily represent the total amount of load inside the joint (118) and it solely divulges information about the loading that takes place in the medial compartments of the knee joint. That said, if biomarker levels are expected to fluctuate according to the degeneration of the articular cartilage that takes place throughout the knee joint as a whole, taking into consideration only the KAM, which only represents the medial loading of the knee, cannot not be fully satisfactory. Nevertheless, the majority of the knee joint load is applied to the medial compartment (117). Further, a number of previous studies examining the relationship between the KAM and disease characteristics relevant to knee OA have provided significant justification for the use of the KAM as a valid proxy for medial compartment knee joint load (19, 91).

For over 40 years the K/L grading scale has been the gold-standard for classifying the severities of OA. Studies have confirmed the reliability and validity of this scale within research and clinical frameworks (119). Scores obtained from Kellgren and Lawrence grading scale are mainly based on formation of osteophytes at edges of the
femur and tibia bones, although it was shown that K/L scale is not strongly dependent on the presence of osteophytes (120). It should also be noted that for investigating the subtle molecular changes that occur inside a knee joint, a five grade scale that is mainly based on the evaluators observations of the osteophytes and JSN, K/L may not provide the most promising means for the purpose of molecular investigation.

4.3 Future Directions

For having a more thorough and inclusive understanding of OA, further research and investigation is certainly necessary. Research that looks into the epidemiological and cultural roots of the diseases can broaden the viewpoint of those who wish to look at the etiology of this disease. Moreover, studies which aim to explore more about the molecular changes in OA need to conduct research with more participants and variables, so that they can detect more factors with influential roles in development and progression of OA. Converging qualitative and quantitative findings can lead clinicians and researchers towards attainment of possible answers about the causation of the disease, contributing risk factors, and biomarkers specific to OA.

In the current study we attempted to clarify the explanatory role of dynamic knee joint load in variance of MMP levels. However, in addition to MMPs, investigating the role of other biomarkers such as cytokines, MMP inhibitors and cleaved parts of Coll-II in OA as well as determining their relationships with each other is important (121). Using such an approach leads to identification of those biomarkers that are more specific to OA and opens the door for detection of new biomarkers. Finding these

60

biomarkers and explaining their variance with currently known OA-related factors, along with risk factors that are thought to be possible OA risk factors, can confirm or reject the validity of the previously detected biomarkers and at the same time can uncover new risk factors that influence the OA status as well as the biomarker levels. It is important to note that including more explanatory variables in a multiple regression model requires larger samples sizes. Ratios between the levels of biomarkers that are attributed to OA can formulate equations that can be indicative of the actual pathophysiological patterns in OA (121).

It is also important to emphasize that biomarkers produced from degeneration of articular cartilage are primarily released into the synovial fluid and the levels detected inside the serum of participants are traces of the actual synovial amounts. Levels of biomarker available in systemic circulation could possibly vary in response to other bodily reactions such as inflammations or tissue remodeling procedures; therefore it can be of great advantage for scientists to collect and analyze synovial fluid samples (113) after attaining the ethics approval. Patients who undergo knee replacement surgeries make good candidates for collection of synovial fluid samples (122); however collecting synovial fluid samples from healthy individuals remains to be problematic.

KAM imp is widely used as a representative of the loading that takes place inside a knee joint, however using KAM has its own limitations. In the current study, five KAM imp values for each knee have been averaged and reported for each knee and the sum of two mean values was reported as the KAM imp Total for every participant. However, the KAM imp Total is not necessarily depicting the actual amount of load that a participant has had in his/her knee joint, in response to which osteoarthritic changes

61

have had occurred throughout the years. KAM imp Total only provides some brief information that depicts the amount of knee joint load in a very small frame of time, and in a controlled environment, while the quantity of intraarticular knee joint load can vary remarkably in actual daily living. Some researchers have looked at the cumulative knee adduction load, which is the product of the KAM imp values and the mean number of steps taken per day (123). The values obtained from cumulative knee adduction load are theoretically closer to the actual amounts of the total load, though it still has certain shortcomings. Finding better substitutes for representing the actual amount of load within the knee joint should be placed in biomechanists' priorities.

Finally, despite being a quite informative tool for clinicians and researchers in classifying different severities of OA, K/L grading scale is not capable of discriminating short-term changes in the stages of the disease, during which articular cartilage undergoes complex pathophysiological changes. Molecular and cellular changes are so fine that discriminating between them according to a 5-grade scale appears to be deficient, nonetheless larger samples sizes may reveal statistical differences even within a 5-grade scale (85). It could be beneficial to produce new OA grading scales with the potency to make distinction between smaller arthritic changes in a more explicit manner.

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Appendix A: Knee Adduction Moment Curves

The following curves demonstrate the knee adduction moment curves from both knees of every participant. OA participant #1 graph shows the KAM curve of the first participant from the OA group, which in tables 3 and 4 was referred to as KOABMK01 (knee osteoarthritis biomarker number 1). The coding of the participants from the OA group was continued in the similar manner and coding of the controls was done using the same strategy, as control participant #1 in the current section is equivalent to CONBMK01 (control biomarker number 1) in tables 2 and 3. Study limb, in the title of each curve, refers to the knee with more OA severity in the OA group and non-study limb is the leg with milder or no OA. For the control group the dominant leg was referred to as the study limb and the non-study limb was the other leg.

The curves illustrate how the knee adduction moment values can fluctuate during the time of each stride. Herein, the five analyzed curves are presented and used for study purposes, so that the area under each curve (KAM impulse) was calculated and the obtained values from the selected trials were averaged for each knee (KAM imp Right/Left). KAM imp from right and left knees were then added together and reported as KAM imp Total, which was the final value used for the purposes of this study.

76
























































Appendix B: Letters of Explanation and Consent Forms

Appendix B1 Blood banking for exploratory future analysis from the: Controls and Biomarkers study

THE UNIVERSITY OF BRITISH COLUMBIA



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Blood banking for exploratory future analysis from the: Controls and Biomarkers study

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|-------------------------|---|
| Co-investigator | Dr. Aziz Ghahary ICORD, the Blusson Spinal Cord Centre |

INTRODUCTION

In addition to the main part of the research study, you are being invited to participate in this additional part. The main part of the research study will examine relationships between specific osteoarthritis (OA) biomarkers (found in blood) and knee joint load. We are asking for you to allow us to keep your blood samples beyond the duration of the main study to allow us to

possibly test novel biomarkers as new information in the research on biomarkers in knee OA emerges.

Your participation in this optional additional blood banking is entirely voluntary, so it is up to you to decide whether or not to take part in this study. Before you decide, it is important for you to understand what the research study involves. Once you have read this consent form and your questions have been answered, you will be asked to sign and date the last page if you want to participate in this part of the study. By signing this consent form, you are stating that you agree freely and voluntarily to participate in this study. You will be given a signed and dated copy of the consent form.

1. <u>WHO IS CONDUCTING THIS STUDY?</u>

This study is being conducted by Dr. Michael Hunt (Principal Investigator) from the University of British Columbia and the Arthritis Research Centre of Canada. Dr. Hunt does not receive any personal financial compensation for this study.

2. <u>BACKGROUND</u>

The influence of biomarkers found in the blood after being released from articular cartilage is an emerging research area. Your banked blood will be a valuable research resource as it will allow us to examine new research ideas as the research area moves forward. For example, if it is suggested that a new biomarker, which can be measured in blood, is an important part of the OA disease process, we can apply for research funding to look at that biomarker in the stored samples from this study, without having to re-do the study.

3. WHAT IS THE PURPOSE OF THE STUDY?

The main goal of banking the blood samples is to allow us to examine biological factors in blood that in the future may be identified as important to the study and treatment of knee OA. The samples will be stored for analyses that are directly related to biological markers relevant to knee OA.

4. WHAT DOES THE STUDY INVOLVE?

This additional part of the main study is asking to continue to keep the blood samples you provided as part of the main study. As part of the main study, you were asked to have blood taken from a vein in your arm. These coded samples will be stored in a freezer in a locked laboratory (in the laboratory of Dr. Aziz Ghahary) for storage for up to 5 years as part of the main study. This additional consent form allows the investigators to store the samples beyond the period of the main study (5 years) for up to 20 years following the end of the main study. Drs. Hunt and Ghahary will be the custodians of the collected samples and will maintain the key to the sample codes on a password protected computer. To ensure that blood samples are used only in valid scientific studies, all future research project requests for the samples will be reviewed by the investigators listed on this consent form and the Clinical Research Ethics Board at UBC.

The blood samples will not be used for commercial purposes and will not be linked to medical data beyond that collected as part of the main study (i.e., walking patterns,).

5. WHAT ARE THE RISKS OF THE STUDY?

There are no anticipated risks of this optional part of the main study. However you do not waive any of your legal rights by signing this consent form.

6. WHAT ARE THE BENEFITS TO TAKING PART IN THIS STUDY?

There will not be direct benefits to you from taking part in this study. We hope that the information learned from this study can be used in the future to benefit other people with a similar disease.

7. <u>WHAT HAPPENS IF I DECIDE TO WITHDRAW MY CONSENT TO</u> <u>PARTICIPATE?</u>

Your participation in this optional part of the main research study is entirely voluntary. Your eligibility to participate in the main part of the study will not be affected by your decision to be part of this blood bank storage or not.

You may withdraw your blood samples from the storage without providing any reasons by writing a request to the principal investigator (Michael Hunt). The samples will then be destroyed. There will be no penalty or loss of benefits to which you are otherwise entitled, and your future medical care will not be affected.

If you choose to withdraw your samples from the storage bank, all data collected about you during the main part of the study will be retained for analysis. By law, this data cannot be destroyed.

8. WHAT WILL THE STUDY COST ME?

You will not be paid for taking part in this optional study. However, there will be no cost to you for this optional part of the main study.

9. <u>CONFIDENTIALITY</u>

Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the Investigator or his or her designate by representatives of Health Canada and the UBC Clinical Research Ethics Board for the purpose of monitoring the research. However, no records that identify you by name or initials will be allowed to leave the investigators' office. Your blood samples will be stored using a unique sample identification number.

Your permission to use and disclose your health information remains in effect until the main study is complete and the results are analyzed. After that time, information that personally identifies you will be removed from the study records. If the results of this study are published or presented in public, information that identifies you will be removed.

10. OUESTIONS

You have read the information in this form. Dr. Hunt or his associates are available to answer your question(s). You know if you have any more questions after signing this you may contact Dr. Hunt or one of his associates at (604) xxx-xxxx.

If you have any questions about your rights as a research subject, you may call the Research Subject Information Line in the University of British Columbia Office of Research Services by e-mail at <u>RSIL@ors.ubc.ca</u> or by phone at 604-822-8598.

SUBJECT CONSENT TO PARTICIPATE

Dr. Hunt (or his associates) has given me information about this research study. They have explained what will be done and how long it will take. They explained any inconvenience, discomfort or risks that may be experienced during this study.

I have read and understood the subject information and consent form.

I have had sufficient time to consider the information provided and to ask for advice if necessary.

I have had the opportunity to ask questions and have had satisfactory responses to my questions.

I understand that all of the information collected will be kept confidential and that the result will only be used for the scientific objectives.

I understand that my participation in this study is voluntary and that I am completely free to refuse to participate or to withdraw from this study at any time without changing in any way the quality of care that I receive.

I understand that I am not waiving any of my legal rights as a result of signing this consent form.

I understand that there is no guarantee that this study will provide any benefits to me.

I have read this form and I freely consent to participate in this study.

I have been told that I will receive a dated and signed copy of this form.

| Printed Name of Subject | Signature | Date |
|--|-----------|------|
| Printed Name of Witness | Signature | Date |
| Printed Name of Principal Investigator/ Designated representative | Signature | Date |

Appendix B2 Biomarkers and knee osteoarthritis: Associations with joint load and effects of exercise

THE UNIVERSITY OF BRITISH COLUMBIA



Department of Physical Therapy Faculty of Medicine 212, Friedman Building 2177 Wesbrook Mall Vancouver, British Columbia V6T 1Z3 Phone: 604.822.8225 Fax: 604.822.1870 Web: www.physicaltherapy.med.ubc.ca

LETTER OF EXPLANATION FOR THE STUDY AND CONSENT FORM

Project Title: Biomarkers and knee osteoarthritis: Associations with joint load and effects of exercise

Principal Investigator: Dr. Michael A. Hunt PhD, PT

Team Members: Dr. Jolanda Cibere MD, MPH, FRCP(C), PhD; Courtney Pollock, MSc, BHScPT

BACKGROUND AND PURPOSE

You are being invited to participate in this study due to your history of knee osteoarthritis (OA). Osteoarthritis is characterized by the breakdown of articular cartilage (a smooth lining found at the ends of bones). The extent of this breakdown has typically been measured using x-rays or magnetic resonance imaging (MRI). Though these methods are successful at assessing the structure of cartilage at a given point of time, measuring changes in cartilage structure over time have proven to be more difficult. This is important when monitoring individuals with knee OA and identifying rates of disease progression. Recent research has identified proteins (biomarkers) that are released into the blood and urine after cartilage breakdown. Importantly, given the direct relationship between these biomarkers and cartilage processes, they may be capable of detecting small changes in cartilage structure. This is especially relevant in the early identification of the disease or when assessing rates of progression at time points sooner than those currently used (usually 12-18 months when using x-rays or MRI). However, little is known about these biomarkers, including: 1) which biomarkers are most associated with cartilage breakdown? and 2) can treatments modify the levels of these biomarkers?

It is well-known that high load placed through the knee joint is a risk factor for articular cartilage breakdown. Sophisticated and non-invasive measurement techniques are able to measure knee joint load during walking and are routinely used in studies of knee OA. We also know that exercise strengthens muscles which act to "offload" cartilage during walking. Therefore, our pilot study aims to answer the above questions by: 1) examining the relationship between various biomarkers and knee joint load during walking, and 2) examining changes in the same biomarkers after an exercise intervention aimed at reducing the load absorbed by knee joint articular cartilage.

WHAT IS A PILOT STUDY?

Before proceeding to a full study, a "pilot study" or "feasibility study" is often carried out first to test the design of a study. The "design" of a study is how the study will be done, how the data are collected, whether that data can provide useful information and whether it will be practical to proceed to a larger study that will include more subjects. This type of study involves only a small number of participants and therefore the results can only be used as a guide for further larger studies. In our case, one of the important questions needed to be answered before moving to a larger study is which particular biomarkers are the most highly related to knee joint load. We are seeking 20 individuals who have knee OA to participate in the study. There is no guaranteed direct benefit to you for participating in this study. It is hoped that additional information gained in this research study may be useful in the treatment of other patients with knee OA.

DETAILS OF THE STUDY

This study has received funding from the Canadian Arthritis Network and involves a series of measurements over an 11-week period. All tests and exercises (if applicable) will take place at the Point Grey Campus of the University of British Columbia.

1) Radiographic assessment of knee OA

We will use x-rays to confirm the presence of knee OA. If you have recent (within 6 months) x-rays of your knee, you can provide us with a copy of the x-ray for analysis. Please note that you will be able to keep your x-ray. If you do not have recent x-rays, you will be referred to have x-rays taken of both of your knees on a date of your choice. We have made arrangements with Laurel Radiology Inc (106-888 8th Ave W, Vancouver) to provide x-ray services and to send us copies of your x-rays. Please note, we will retain copies of all x-rays that are taken at Laurel and will store them in a locked filing cabinet in our laboratory. You will not be required to pay for these x-rays, but you will be required to pay for any associated travel and/or parking costs.

2) Description of the testing sessions

If you pass radiographic screening, you will undergo further measurement testing on two occasions (session #1 at baseline – start of the study, and session #2 at follow-up – end of the study). It is expected that these sessions will last approximately 3-4 hours each. The following tests will be performed at **both** sessions:

a) Collection of blood and urine samples:

You will report to the UBC Laboratory at UBC Hospital (main floor) where you will have a small amount of blood drawn and be asked to provide a urine sample. In order to ensure steady amounts of biomarker levels, you will be required to remain seated for at least 30 minutes prior to having the blood drawn and urine collected. Your samples will be placed in sterile receptacles that will only be identified by a protected code only accessible by Dr. Hunt and the study coordinator. All subsequent handling and analysis of samples will include special identifying

codes, again only accessible by Dr. Hunt and the study coordinator. There will be no obvious identifiers on the receptacles that will link any information about you to the samples.

b) Measurement of physical function, walking patterns, and muscle strength:

Immediately upon completion of blood and urine collection, you will be met by the study coordinator. You will then proceed to Dr. Hunt's Motion Analysis and Biofeedback Laboratory, which is located on the 3rd floor of UBC Hospital. During the testing session in the lab, you will undergo the following examinations:

(i) assessment of your physical function. You will be asked to climb up and down a flight of 10 stairs which will be timed with a stopwatch. This will be completed twice in succession, though you will be given a rest period between trials.

(ii) assessment of your walking patterns. You will walk barefoot and wearing shorts and a special t-shirt with 2 holes cut out. Reflective skin markers will be attached to your skin at various sites such as the ankle, knee and hip (additional markers will be placed under your chin and on your back within the holes cut into the t-shirt). You will be filmed with special cameras that track the movement of the reflective markers as you walk along a walkway. The movement of the markers enables a recreation of a moving "stick figure" on the computer screen that mimics your movements. The image in no way provides any information about your unique coded identifier (ie. not your name). From the walking tests, we will be able to analyze the movements at your hip, knee and ankle, as well as the forces acting across each joint. You will complete approximately 10 trials of walking at your own pace, and each trial will be 10m in length.

(iii) assessment of muscle strength. The maximal strength of your front and back thigh muscles and your hip muscles will be measured while sitting using a special machine in which you are strapped into. Your hip muscles will be measured while standing as you push into the same device. You will be asked to push up against an ankle pad as hard as you can three (3) times for each muscle group.

You must report to the investigator any pain or discomfort during any of the testing procedures. As noted above, you will be required to wear shorts and a t-shirt during all testing. You may either bring your own shorts or we can provide you with some.

c) Self-report questionnaires of pain and physical function:

You will be provided with questionnaires to be completed 1-2 days prior to your testing sessions at UBC. These questionnaires will be 11-12 pages in length and will ask you questions regarding the extent of your knee pain, the magnitude of difficulty in performing certain physical tasks, and the magnitude and types of physical activity in which you participate. If you have any questions about the questionnaires, feel free to ask the study coordinator upon presentation to the laboratory for testing. Further, please note that you do not need to answer any questions which you are not comfortable answering.

3) Description of the exercise program

After you have completed the tests, you will be randomly assigned to either an exercise group or a control group for the study. There is a 50% chance of being allocated to either group. If you are allocated to the control group, you will not be given any exercises and you will be asked to

maintain your usual physical activity and refrain from commencing any exercise program for the duration of the study (11 weeks). If you are allocated to the exercise group, you will be given lower limb muscle strengthening exercises to do at home 4 days a week for 10 weeks. You will also report back to UBC for visits with Dr. Hunt who will teach you the exercises and give you ankle weights and a rubber band to take home. These sessions will occur once per week at weeks 1, 2, 3, 5, and 8 (a total of 5 sessions) to check and progress your exercises. You will also be given a training diary to record how often you do your exercises and to monitor your symptoms and medications at regular intervals. At the end of the study 11 weeks later, all participants (control and exercise group) will report back to UBC Hospital for the same tests that were undertaken at the start of the study.

PARTICIPATION CRITERIA:

You are invited to participate if you are greater than 50 years of age and have had medial (inside) knee pain on most days of the previous month (rated as greater than 3 out of 10 on a scale from 0 "no pain" to 10 "maximal pain"). You are not eligible if you are currently in a structured exercise program; if you have a medical condition precluding exercise; if you have inflammatory arthritis (e.g. rheumatoid arthritis or gout) in any joint; if you have previously had a hip or knee replacement surgery; if you have recently (within the past 6 months) had corticosteroid (injection or oral) treatment or arthroscopic knee surgery; if you have significant hip or back pain; if you are unable to attend both testing sessions and all 5 training sessions (if randomized to the exercise group) at UBC; if your pain comes predominantly from under your kneecap; or if you are unable to walk without the use of a gait aid. Finally, if your x-rays show that your OA is predominantly in the lateral (outside) compartment, you are ineligible to participate in this study.

RISKS/SIDE-EFFECTS:

If you are required to have x-rays as part of this study, you will be exposed to radiation as part of this process. Ill effects of exposure to very high doses of radiation have been documented (such as increased cancer rates). We will be using standard knee x-rays that are used every day for clinical purposes. Therefore, the amount of radiation that you would be exposed to will be no more than that expected from standard clinical care.

You may experience increased knee or hip pain while you are performing the strength measures or the walking assessment or the pain may be felt the next day. You should notify the tester if this occurs. You may also experience some mild skin irritation from the reflective markers placed on your skin or if shaving is required prior to marker placement. If this is bothersome, you should inform the tester.

If you are in the exercise group, it is also possible that you may experience increases in knee, hip or back pain with the exercises, especially at the beginning of the program. To minimise this, you will be given clear instructions by Dr. Hunt (a licensed physical therapist) to gradually increase your exercises. You should telephone Dr. Hunt if your pain increases or does not subside within 2 or 3 days. Signing this consent form in no way limits your legal rights against the sponsor, investigators, or anyone else.

In the unlikely event of a medical emergency during any of the assessments or exercise sessions, the research personnel will call 911.

BENEFITS:

Although it is possible that you may not receive any personal benefit from your participation, the findings from this study may contribute to our understanding of the assessment and treatment of knee OA.

REIMBURSEMENT:

We appreciate your involvement in this study and do not want you to incur any cost associated with travel to the laboratory. We will provide parking adjacent to the building where the testing and training (if applicable) will take place. If you prefer to take public transit, we will provide you with return bus fare to/from your residence. Please retain your receipts.

CONFIDENTIALITY:

Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the Investigator or her designate by representatives of Health Canada, and the UBC Research Ethics Board for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the Investigators' offices. Your individual results will be held in confidence. No person other than the investigators will be given access to your records without your expressed permission. When the results are reported, individual records will be coded or reported as group data. Representatives of The University of British Columbia Research Ethics Board may require access to your study-related records to monitor the conduct of the research. This Board aims to help protect the rights of research subjects. If you would like a copy of the study results, please indicate this on the consent form.

VOLUNTARY PARTICIPATION:

Participation in the study is completely voluntary. You may refuse to participate or withdraw from the study at any time with no effect on you, including clinical care. You are not obligated to provide any reason for your withdrawal, should you choose to do so. Participation in this study does not prevent you from participating in other research studies in the future. If you are willing to be contacted in the future for other research studies, please indicate this on the consent form. You can always withdraw this consent to be contacted in the future, should you change your mind. In this case, your name and contact information will be removed from our records. Participation in any future research is completely voluntary and will have no bearing on the results of the current research project.

FURTHER QUESTIONS?

Please contact one of us, at the address below or by phone, to ask any questions you may have about the study.

| Principal Investigator: | Co-investigator: | Graduate Student: |
|---------------------------|--------------------------------------|------------------------------|
| Michael A. Hunt PhD, PT | Jolanda Cibere MD, MPH, FRCP(C), PhD | CourtneyPollock, MSc, BHScPT |
| Assistant Professor | Assistant Professor | Doctoral Student |
| Dept. of Physical Therapy | Dept. of Rheumatology | Rehabilitation Sciences |
| 2177 Wesbrook Mall | Arthritis Research Centre of Canada | 2177 Wesbrook Mall |
| 212, Friedman Building | 300-895 West 10 th Ave. | 212, Friedman Building |
| Vancouver, BC | Vancouver, BC | Vancouver, BC |
| V6T 1Z3 | V5Z 1L7 | V6T 1Z3 |
| 604-xxx-xxxx | 604-xxx-xxxx | 604-xxx-xxxx |

If you have any concerns about your treatment or rights as a research subject, you may contact the Research Subject Information Line in the UBC Office of Research Services at 604-822-8598 or if long distance e-mail to <u>RSIL@ors.ubc.ca</u>. Please keep this information letter for future reference.

THE UNIVERSITY OF BRITISH COLUMBIA



Department of Physical Therapy Faculty of Medicine 212, Friedman Building 2177 Wesbrook Mall Vancouver, British Columbia V6T 1Z3 Phone: 604.822.8225 Fax: 604.822.1870 Web: www.physicaltherapy.med.ubc.ca

CONSENT FORM

Biomarkers and knee osteoarthritis: Associations with joint load and effects of exercise

I have read the Letter of Explanation, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction. I have been told that I will receive a signed and dated copy of this consent form for my own records. My signature indicates that I consent to participate in this study.

| Print Name | Signature | Date |
|--|-----------|------|
| Signature of Person Obtaining Consent: | | |

Print Name

Signature

Date

Possibility of future research

There may be future opportunities for you to participate in ongoing research. If you are interested in being contacted, please check the appropriate box below. If contacted, you will be asked to read a new letter of information and sign a new consent form.

 \Box Please do not keep my name and contact information. I do not wish to be contacted in the future.

□ Please keep my name and contact information so that I may be contacted to learn about future research opportunities or have access to my data in the future.

Copy of Study Results

I would like a copy of the study results. Yes \Box No \Box If yes, please write your mailing address below.

Appendix B3 Functional mobility control database

THE UNIVERSITY OF BRITISH COLUMBIA



Department of Physical Therapy Faculty of Medicine 212, Friedman Building 2177 Wesbrook Mall Vancouver, British Columbia V6T 1Z3 Phone: 604.822.8225 Fax: 604.822.1870 Web: www.physicaltherapy.med.ubc.ca

INFORMED CONSENT FORM

Project Title:Functional Mobility Control DatabasePrincipal Investigator:Dr. Michael A. Hunt PhD, PTCo-investigator:Dr. S. Jayne Garland PhD, PTJudit Takacs MSc, PhD studentJerrad Guenther BSc, Research CoordinatorMohammadreza Bahar PT, MSc studentCourtney Pollock BHScPT, MSc, PhD student

Contact information:

| Principal Investigator: | Graduate Students: |
|---------------------------|---|
| Michael A. Hunt PhD, PT | Judit Takacs, Mohammadreza Bahar Courtney Pollock |
| Assistant Professor | Graduate Student |
| Dept. of Physical Therapy | Rehabilitation Sciences |
| 2177 Wesbrook Mall | University of British Columbia |
| 212, Friedman Building | 2211 Wesbrook Mall |
| Vancouver, BC | Vancouver, BC |
| V6T 1Z3 | V6T 1Z3 |
| (604)xxx-xxxx | (604)xxx-xxxx |

BACKGROUND AND PURPOSE

We aim to gather information regarding the movements and joint loading as well as muscle activation patterns at the hip, knee and ankle during functional mobility in individuals. We will use this information to compare healthy individual's performance with that of those with musculoskeletal (muscles, bones and/or joints) and/or neurological (involving the nervous system) impairments which impact functional mobility. We also wish to measure the levels of proteins found in the blood that result from the breakdown of joint cartilage. This information will be used to compare levels in people with and without knee osteoarthritis. We hope that this protein comparison will provide new information needed to develop methods of diagnosing osteoarthritis earlier.

You are being invited to participate in this study that will measure your functional mobility including your joint movement patterns and muscle activation patterns, during walking and functional tasks such as fast stepping, squats, lunges, and jumping as well as the strength of your hip, knee and ankle muscles. We will also measure your balance during walking.

DETAILS OF THE STUDY

Prior to commencing the functional assessment a screening for radiological evidence of knee osteoarthritis (OA) is required. If you have recent (within 6 months) x-rays of your knee, you can provide us with a copy of the x-ray for analysis. Please note that you will be able to keep your x-ray. If you do not have recent x-rays, you will be referred to have x-rays taken of both of your knees on a date of your choice. We have made arrangements with False Creek Diagnostic Imaging (555 8th Ave W, Vancouver) to provide x-ray services and to send us copies of your x-rays. Please note, we will retain copies of all x-rays that are taken at Laurel and will store them in a locked filing cabinet in our laboratory. You will not be required to pay for these x-rays, but you will be required to pay for any associated travel and/or parking costs.

Approximately one to two weeks after receiving your x-ray images, you will undergo further measurement. It is expected that this session will last approximately 2-3 hours on one day at the Point Grey Campus of the University of British Columbia in Dr. Hunt's Motion Analysis and Biofeedback Laboratory, which is located on the 3rd floor of UBC Hospital. During a testing session in the lab the following measurements will be collected:

1) Collection of blood samples:

If your x-rays were found to be clear of any evidence of knee OA, you will be asked to provide a sample of blood for protein analysis. A member of the lab will accompany you to the UBC Outpatient Laboratory at UBC Hospital (main floor) where you will have a small amount of blood drawn. Your samples will be placed in sterile receptacles that will only be identified by a protected code only accessible by Dr. Hunt and the study coordinator. All subsequent handling and analysis of samples will include special identifying codes, again only accessible by Dr. Hunt and the study coordinator. There will be no obvious identifiers on the receptacles that will link any information about you to the samples. Note that if your x-ray image does show evidence of knee OA, you will be ineligible for the analysis of blood proteins, however, you will still be eligible to undergo all testing described below.

2) Maximal lower extremity muscle strength:

We will use handheld dynamometry and isokinetic measurement using (Biodex) to measure your hip, knee and ankle strength. A handheld dynamometer is a device that fits in the palm of one's hand that measures the force of a body segment pushing against it. A Biodex is a seated strength test device that measures the force of the body segment pushing against the lever of the machine, which is positioned specifically for each joint of the body. For our study, we will test the strength of your hip flexors (front of hip), extensors (back of hip), abductors (outside of hip), adductors (inside of hip), hip rotators (inside and outside of thigh), knee flexors (back of thigh), knee extensors (front of thigh), ankle dorsiflexors (front of foot) and ankle plantarflexors (back of ankle). You will be asked to push as hard as you can with your leg against the dynamometer or Biodex, three times (about 5 seconds each time) for each muscle group. You will be given plenty of rest between each trial to minimize the effect of fatigue.

3) Three dimensional functional mobility analysis:

You will be asked to walk and perform functional tasks such as walking, fast stepping, squats, and lunges. Note that during the fast stepping tasks, you will be provided with the option of using a safety harness designed to prevent falls. For each task you will be barefoot and wearing shorts and a special t-shirt with 2 holes cut out. Reflective skin markers will be attached to your skin at various sites such as the ankle, knee and hip (additional markers will be placed under your chin and on your back within the holes cut into the t-shirt). We will also measure muscle activity of your hip, knee and ankle muscles using electromyography. Electromyography is the measurement of the electrical activity in your body that causes your muscles to work. This requires the attachment of electrodes to your skin around your hip, knee and ankle joint (similar to how an electrocardiogram measures the electrical activity of your heart). These electrodes only measure the muscle activity and do not provide any electrical stimulation to you. You will also have the activity of your heart (e.g. heart rate) monitored with the use of a three electrode heart rate sensor.

You will be filmed with special cameras that track the movement of the reflective markers as you walk along a walkway and perform the functional tasks as noted above. The movement of the markers enables a re-creation of a moving "stick figure" on the computer screen that mimics your movements. Digital video cameras will also be used to record your movements. This video data will be stored on password-protected computers and assigned a unique identifying code that in no way reveals your identity. These video images will not be used in any other form without your explicit consent to do so. From the functional mobility tests, we will be able to analyze the movements at your trunk, hip, knee and ankle, as well as the forces acting across each joint. You will complete approximately 5 trials of each functional task with rest as required and all activities will be performed at your own pace.

4) You will be asked to complete questionnaires to assess any discomfort you have experienced with activities you may perform throughout a typical day, daily activity levels, quality of life and concerns regarding falling during activities performed on a typical day. You do not have to answer any questions which you do not feel comfortable answering.

5) Your balance will be measured during a variety of activities with scales such as the Berg Balance Scale and the Community Balance & Mobility Scale. These scales measure balance performance related to standing and walking balance required for community mobility (e.g. rising from sitting, walking and turning your head, walking and picking up an object from the ground, walking and carrying bags, standing on one leg). You will also complete one standardized test of function called the Time-Up-and-Go test. This test will measure the time it takes for you to rise from a chair, walk 3 meters, turn around, walk back and sit down again.

You must report to the investigator any pain or discomfort during any of the testing procedures. As noted above, you will be required to wear shorts and a t-shirt during all testing. You may either bring your own shorts or we can provide you with some.

PARTICIPATION CRITERIA:

You are invited to participate if you are between the ages of 45 and 80 and have no musculoskeletal or neurological impairments which impact your functional mobility. You must also have no history of significant lower body injuries or conditions that would impair the measurement of walking or hip, knee or ankle strength, no history of significant neurological injury that affects your walking, no diagnosis of arthritis in the hip, knee or ankle, and no history of, or planned, lower limb joint replacement surgery.

RISKS/SIDE-EFFECTS:

If you are required to have x-rays as part of this study, you will be exposed to radiation as part of this process. Ill effects of exposure to very high doses of radiation have been documented (such as increased cancer rates). We will be using standard knee x-rays that are used every day for clinical purposes. Therefore, the amount of radiation that you would be exposed to will be no more than that expected from standard clinical care.

You may experience some mild skin irritation from the reflective markers or electrodes placed on your skin or if shaving the skin is required prior to marker/electrode placement. You may also experience some mild muscle discomfort during the muscle strength testing. Additionally, you may experience fatigue during testing. If this is bothersome, you should inform the tester immediately.

Signing this consent form in no way limits your legal rights against the investigators, or anyone else.

In the unlikely event of a medical emergency during the assessment, the research personnel will call 911.

BENEFITS:

You will not receive any direct benefit from participation in this study. However, the findings from this study may contribute to our understanding of the effects of neuromusculoskeletal impairments on walking patterns, blood proteins, and muscle strength related to functional mobility. Your data will remain de-identified and no researcher not listed on this form will have access to any confidential information.

REIMBURSEMENT:

You will not be reimbursed for your involvement in this study.

CONFIDENTIALITY:

Your confidentiality will be respected. No information that discloses your identity will be released or published without your specific consent to the disclosure. However, research records and medical records identifying you may be inspected in the presence of the Investigator or her designate by representatives of Health Canada, and the UBC Research Ethics Board for the purpose of monitoring the research. However, no records which identify you by name or initials will be allowed to leave the Investigators' offices. Your individual results will be held in confidence. No person other than the investigators will be given access to your records without your expressed permission. When the results are reported, individual records will be coded or reported as group data. This study has been approved by the UBC Clinical Research Ethics

Board. This Board aims to help protect the rights of research subjects. If you would like a copy of the study results, please indicate this on the consent form.

VOLUNTARY PARTICIPATION:

Participation in the study is completely voluntary. You may refuse to participate or withdraw from the study at any time with no effect on you. You are not obligated to provide any reason for your withdrawal, should you choose to do so. Participation in this study does not prevent you from participating in other research studies in the future. Further, you may ask to have your data permanently removed from our database at any time without penalty. If you are willing to be contacted in the future for other research studies, please indicate this on the consent form. You can always withdraw this consent to be contacted in the future, should you change your mind. In this case, your name and contact information will be removed from our records. Participation in any future research is completely voluntary and will have no bearing on the results of the current research project.

FURTHER QUESTIONS?

If you have any concerns about your experience or rights as a research subject, you may contact the Research Subject Information Line in the UBC Office of Research Services at 604-822-8598 or if long distance e-mail to <u>RSIL@ors.ubc.ca</u>. Please keep this information letter for future reference.

Principal Investigator: Graduate Students: Judit Takacs, Mohammadreza Bahar or Courtney Pollock Michael A. Hunt PhD, PT Assistant Professor Graduate Student Dept. of Physical Therapy **Rehabilitation Sciences** 2177 Wesbrook Mall University of British Columbia 212, Friedman Building 2211 Wesbrook Mall Vancouver, BC Vancouver, BC V6T 1Z3 V6T 1Z3 (604)xxx-xxxx (604)xxx-xxxx



Department of Physical Therapy Faculty of Medicine 212, Friedman Building 2177 Wesbrook Mall Vancouver, British Columbia V6T 1Z3 Phone: 604.822.8225 Fax: 604.822.1870 Web: www.physicaltherapy.med.ubc.ca

CONSENT FORM

Functional Mobility Control Database

I have read the Letter of Explanation, have had the nature of the study explained to me and I agree to participate. All questions have been answered to my satisfaction. I have been told that I will receive a signed and dated copy of this consent form for my own records. My signature indicates that I consent to participate in this study.

| Print Name | Signature | Date |
|--------------------------------------|-----------|------|
| Principle investigator or designate: | | |
| | | |
| Print Name | Signature | Date |
| Possibility of future research | | |

Possibility of future research

There may be future opportunities for you to participate in ongoing research. If you are interested in being contacted, please check the appropriate box below. If contacted, you will be asked to read a new letter of information and sign a new consent form.

Please keep my name and contact information so that I may be contacted to learn about future research opportunities or have access to my data in the future.

Copy of Study Results

I would like a copy of the study results. Yes No If yes, please write your mailing address below.