I, Rebecca J Nesbitt, hereby submit this original work as part of the requirements for the degree of Doctor of Philosophy in Biomedical Engineering.

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Establishing Design Criteria for Anterior Cruciate Ligament Reconstruction

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Abstract

The anterior cruciate ligament (ACL) plays a complex role in knee stability. Injury to this structure can cause abnormal joint kinematics and loadings which may lead to the early onset of osteoarthritis (OA) and joint degeneration. While surgeons are able to restore joint laxity in the short to medium term, long term OA development is currently not prevented in patients who have undergone surgical repair. In order to slow or stop the progression of OA following ACL injury, we hypothesize that reconstruction techniques must achieve a greater degree of native ligament functionality.

The principles of Functional Tissue Engineering state that a ligament’s functionality may be defined as its in vivo loading characteristics. While this information remains impossible to measure directly in human patients in vivo, in vitro testing can serve as an alternative as long as the following conditions are met: 1) Loads are measured in 6 degrees-of-freedom (DOFs); 2) Loads are measured during activities of daily living (ADLs); 3) Loads are measured within a realistic environment, which may include knees sustaining injury to structures influencing ACL functionality.

Due to the invasive nature of in vivo load sensing, researchers have turned to robotics to simulate ADLs kinematics on biological tissue. This technique allows open access to the joint to measure contact forces and 6 DOF ligament loads throughout physiologic motion paths, fulfilling the first 2 requirements for in vitro testing. By using an animal model, specimen-specific kinematics may be collected and applied to the same tissue, overcoming several limitations of cadaveric testing, including specimen quality and kinematic mis-matches. It also allows for consideration of biologic effects and controlled testing of various knee pathologies, fulfilling the 3rd requirement for in vitro testing.
Because of these advantages, this work utilized robotics in combination with the sheep knee model to study in vivo ACL loading, which may then serve as design criteria for new and novel repair techniques. Studies were designed to address two specific aims.

The first focused on assessing the biomechanical relationships between activity and the corresponding demands placed on the ACL. Results showed that, while a strong link exists between activity and the corresponding knee kinematics, the knee dynamics follow a more complex pattern with inter-relationships between multiple DOFs. Overall, ACL functional demands were most variable during phases of the activities when the knee was less weight bearing, yet still engaged. Specifically, inclined gait placed higher demands on the ACL during hoof strike while declined gait place higher demands on the ACL during push off. Both of these time points corresponded to instances of lower compression levels within each ADL. This is also consistent with the timing of non-contact ACL tears, where most injuries occur during the transition from uncompressed to compressed knee states – such as landing.

The second aim focused on assessing biomechanical relationship between ACL demands and concomitant knee injury. Medial meniscus (MM) injury increased ACL forces during the transitions between swing and stance in response to significant increases in anterior translation. Dual (MM and MCL) injury produced no increases, yet both MM and Dual groups developed significant OA within the medial compartment. MCL injury produced increased ACL force during mid stance in response to increased overall joint laxity but no increase in OA. Results of the this study are the first to relate ADLs and injury of surrounding structures to resulting knee biomechanics and ACL function and provide preliminary data for defining design requirements for future ACL reconstruction techniques.
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Chapter 1

Background, Rationale, and Objectives

ACL Injury Incidence and Significance

The anterior cruciate ligament (ACL) is essential to maintaining knee stability. It functions as the primary restraint to anterior tibial translation and as a secondary restraint to adduction-abduction (or varus-valgus) and internal-external rotations\(^1\)-\(^3\). It is one of the most commonly injured structures of the knee, with most injuries occurring during athletic activities\(^4\). There are estimates of up to 250,000 ACL injuries occurring in the United States each year\(^5\), accounting for approximately 8% of all soft tissue injuries. While both male and female athletes are affected\(^6\), female athletes are up to 10 times more likely to experience injury for each athletic exposure\(^4\). Following ACL injury, the knee possesses a higher degree of laxity. This laxity causes abnormal knee kinematics, which can degrade articular cartilage over time, leading to joint morbidity and pain\(^3,\(^7\). To avoid this outcome, surgical reconstruction of the ACL has become the standard for treatment, with the goal of restoring the structure and function of the native ligament\(^8\). With such proactive treatment standards, ACL injuries cost the US economy billions of dollars each year\(^9\)-\(^12\), with 100,000 ACL reconstructions performed annually\(^13,\(^14\) at an estimated $17,000 per procedure\(^9,\(^10,\(^15\).

Current ACL Reconstruction Methods

As the primary restraint to anterior translation\(^1\), the surgeon’s goals for ACL reconstruction are to restore knee stability, return patients to daily activities with pain reduction, and prevent long-term joint degeneration\(^3\). The first reconstructions consisted of surgeons directly suturing the
failed ends of the torn ligament together\textsuperscript{16-22}. However, due to its high failure rate, this method is now rarely employed\textsuperscript{23}. The limited blood supply within the knee, a synovial joint, causes the ACL to have a very poor healing capacity\textsuperscript{24}. Therefore, total ligament reconstruction has since been adopted as the preferred treatment for ACL injury\textsuperscript{13}. An ACL reconstruction replaces the damaged ligament with a graft which can be made of natural or synthetic material. Common graft selections include the combined semitendinosus and gracilis autograft or the “gold standard” bone-patellar tendon-bone (BPTB) autograft\textsuperscript{13,25}. While the single bundle BPTB autograft has demonstrated the ability to restore antero-posterior (A/P) kinematics in the short term\textsuperscript{3,13,25}, Tashman et.al has shown that A/P translation and medial-lateral laxity increases over time and fails to restore both internal-external and varus-valgus rotational kinematics\textsuperscript{3,26}. These changes in knee kinematics are believed to shift cartilage contact regions to areas not accustomed to loading during activities of daily living (ADLs). This could trigger mechanical and/or biological catalysts which are believed to be responsible for progressive cartilage degeneration and the early onset osteoarthritis (OA)\textsuperscript{3,26}. Recent studies of long-term outcomes for repairs indicate that between 50-85\% of patients experienced early symptoms of OA within 11-20 years of surgery\textsuperscript{27-31}.

This joint degeneration strongly implies that the functional requirements of the native ACL are not being met in patients undergoing reconstruction. The only way to improve injury prognosis and better replicate normal ligament function is to build on our understanding of the complex role of the ACL by studying it during ADLs\textsuperscript{32}. Such physiologically relevant research is critical to develop functionally-based design criteria against which we can benchmark future repair techniques to improve surgical outcome and patient quality of life.
**Rationale and Objective**

This dissertation encompasses the NIH R01 grant entitled “Establishing Design Criteria and Large Animal Model for Evaluation of ACL Repair”, whose purpose is to set design criteria and evaluation benchmarks for both existing and novel ACL treatment strategies such as tissue engineered repairs. In addition to achieving the stated aims of the grant, this work also provides critical information needed to further improve our robotic testing platform and to translate findings from a large animal model to improving ACL reconstruction in human patients. Outcomes of this work will supply the first published knee kinetics directly measured in situ for individualized activities of daily living, as recorded in vivo. As current research has relied heavily on computational methods and non-physiologic testing scenarios to generate estimates of native loading, these results will provide the crucial link back to the real and relevant question surrounding ACL repair: What, and in which directions, are the functional loading demands of the ACL during activities of daily living?

Only by answering this question can researchers address the severe consequences surrounding an ACL injury, which include drastic decreases in activity, increased pain, and continued financial burden. Each year, 1 in every 3000 people will experience a full or partial ACL tear. The loss of ACL function dramatically alters knee kinematics and joint contact pressures and affects the surrounding knee joint structures. This change in biomechanics can lead to further joint problems. Patients have been shown to be at an increased risk of developing OA earlier in life, even when treated with surgical reconstruction or conservatively through rehabilitation and activity modification. To re-establish the knee’s native biomechanics and avoid degeneration, there is a need to establish design criteria to benchmark potential repair strategies aimed at restoring true ACL functionality.
In ACL research, we define true functionality based on the principles of functional tissue engineering\textsuperscript{35}, which describe tissue according to functional parameters of the in vivo condition. Thus, the functional tissue engineering parameters (FTEPs) of the ACL are centered on the structure’s ability to transmit load across the knee. To meet the demands of daily life, researchers must define these ACL FTEPs under physiologic conditions that a reconstructed knee will likely experience. This includes varying levels of activity and potential concomitant injuries to surrounding knee structures. However, in vivo ACL loads have yet to be accurately quantified, and the influences of different activities of daily living (ADLs) and injury to surrounding structures remain unknown. Due to the invasiveness of in vivo force sensing in human subjects, studies in the previously established sheep model\textsuperscript{36} are an attractive alternative to examine these factors.

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*Table 1.1.* List of acronyms used throughout.
Animal models allow researchers to quantify biomechanical metrics in vivo and to simulate conditions in vitro where we can gain access to tissues to more accurately determine FTEPs. Combining technologies from implantable electromagnetic (EM) trackers and robotics allows us to reproduce in vivo kinematics for individualized, in vitro simulations of in vivo loading conditions. Using this methodology, physiologic conditions relevant for establishing FTEPs were simulated via a series of studies in the sheep model to examine activity and injury related changes in 1) vertical ground reaction forces (VGRFs), 2) three-dimensional (3D) knee kinematics, 3) joint contact pressures, 4) total knee loads, 5) ACL loads, and 6) articular cartilage health.

Studies were designed to answer three research questions: 1) How does altering gait surface inclination in the normal sheep knee affect the first 5 response measures? 2) How does MCL injury or medial meniscectomy (MM) affect all 6 measures? 3) How does combining these two injuries (Dual) alter all 6 response measures compared to those for both individual injuries? By answering these questions, we gained insight into the control mechanisms behind knee biomechanics and drive the creation of reconstruction requirements. Studies were broken up in support of two overarching aims:

**Specific Aims**

**Specific Aim 1:** Examine how, compared to level gait, uphill and downhill gait alters normal knee VGRFs, 3D knee kinematics, knee contact pressures, total knee loads, and ACL loads.

**Rationale:** The impact of different activities of daily living on ACL force has yet to be directly investigated. At best, researchers have only hypothesized how directional changes in in vivo kinematics may affect ACL force in a single direction. By reproducing in vivo kinematics of three distinct ADLs, we may begin to interpret the relationships between VGRFs, 3D kinematics, knee
contact pressures, and 6 degree-of-freedom (DOF) total knee and ACL loads. Understanding these biomechanical relationships will allow future studies to specifically target high risk ADLs and customize ACL repairs to meet the needs of individual patients and activity levels.

Chapter 3 directly addresses this aim in the sheep model through a part in vivo and part in vitro study. All metrics listed above were measured and compared across each ADL. Chapter 4 supports this aim by testing the accuracy of our measurement system and assesses risks for similar cadaveric studies simulating higher intensity ADLs. Chapter 5 compares and contrasts biomechanics of sheep and human cadaveric models during an identical ADL to validate findings using the sheep model. Chapter 6 investigates the challenges of applying standardized ADLs to a cadaveric population which contains inherently high population variability.

**Specific Aim 2:** Examine how, compared to normal knees, MCL and/or MM injury alters normal knee VGRFs, 3D knee kinematics, knee contact pressures, total knee loads, ACL loads, and articular cartilage health.

**Rationale:** With the majority of early onset OA occurring in the medial compartment of the knee, special treatment may be warranted when treating ACL reconstruction patients with concomitant injuries to the medial structures. By investigating the specific regional impacts of these structures on articular cartilage health and identifying the key biomechanical metric(s) leading to the condition, we may prioritize ACL reconstruction design criteria to minimize cartilage damage in dual-injured patients.
Chapter 7 supports this aim by quantifying the amount of biomechanical change that occurs in response to an MCL, MM, or Dual injury compared with pre-injury values. Chapter 8 directly addresses Aim 2 in the sheep model through a part in vivo and part in vitro study after 12 weeks of healing. All metrics listed above were measured and compared across each ADL and across each injury group.

Results of these 2 aims represent baseline design criteria for potential ACL repair techniques in the sheep model, and provide valuable insight into how ADLs and/or injury to surrounding structures can alter the functional requirements of an ACL repair in human patients.
Chapter 2

Literature Review

ACL Anatomy and Function

The anterior cruciate ligament (ACL) is one of a pair of “crossing” (i.e. cruciate) ligaments located in the intra-articular space of the knee joint. It is a viscoelastic band of connective tissue surrounded by a synovial membrane\(^8,24,37,39\). Its longitudinal axis is composed of parallel bundles of type I collagen interspersed with type III collagen reticular fibers\(^8,24,38\). In the context of human developmental biology, it begins to appear between six and eight weeks of gestation but is not fully developed until week 14\(^8,38\). The middle geniculate artery serves as its main blood supply\(^24,38\). However, blood vessels within the ACL are not homogenously distributed. The distal portion of the ligament has been shown to have a smaller blood supply and contains an avascular fibrocartilaginous area\(^24,38\), which likely plays a role in its poor healing capacity\(^24,38\). While there are relatively few neural receptors within the total volume of the ACL, the mechanoreceptors that do exist contribute significantly to proprioception pathways of joint position\(^40\).

Figure 2.1. Anterior view of the gross anatomy of right side human knee.
The ACL originates from the medial portion of the inner lateral femoral condyle and inserts into the anterior aspect of the intercondylar fossa of the tibial plateau between the tibial spines (Figure 2.1). Both origin and insertion sites are regarded as oval shaped, with the femoral footprint measuring approximately 11 mm along the antero-posterior direction, and 18 mm along the proximal-distal direction. Similarly, the tibial footprint measures approximately 11 mm along the medial-lateral direction by 17 mm along the antero-posterior direction. Each of these footprints can be up to 3.5 times larger than the cross-sectional area of the ACL midsubstance. The average dimensions of the ACL are 32 mm long, by 10 mm wide, by 5 mm thick.

Because native ACL forces cannot be directly measured in vivo without physically altering the ACL itself, investigators have attempted to measure its functional demands by performing cadaveric studies. Previous studies have shown that its anatomic position and complex structure make the ACL a major stabilizer in restraining anterior tibial translation, internal rotation, and varus-valgus angulation. This diverse function is thought to be promoted by the existence of two distinct bundles, the anteromedial (AM) and posterolateral (PL), which twist and untwist around one another throughout the knee’s range of motion (Figure 2.2). The anteromedial bundle is believed to remain nearly isometric with a length change of only about 2 mm over the range of knee motion, whereas the posterolateral bundle increases in length as the knee extends. While ACL force has been shown to peak between 15° and 30° of flexion and decrease with flexion angle, force magnitude also depends on activity level. For example, Li et al. estimates that a 200 N quadriceps force creates ACL forces of 72 ± 28 N at 30° flexion but only 10 N at 90° flexion. Such complexity makes it essential to
quantify native loads in all directions and at multiple points during an activity to fully describe functionality. Yet, researchers typically are only able to study ACL force in a fixed knee position along a single loading direction\textsuperscript{51,52,54-61}. As a result, current ACL force data is rarely representative of actual in vivo conditions.

**Figure 2.2.** Interactions of the AM and PM bundles of the ACL change with flexion angle, creating multiple directions of loading.

In addition to inherent complexity, the impacts of surrounding knee structures on the ACL also plays a role in its poorly defined function. Approximately 13\% of patients with an ACL injury also sustain a dual medial meniscus injury, and about 30\% sustain a dual medial collateral ligament (MCL) injury\textsuperscript{62}. Both structures are frequently left unrepaired. Cadaver studies have shown that the MCL and medial meniscus resist passive varus-valgus and internal-external rotation, indicating that their absence may influence ACL function\textsuperscript{63-66}. Ma et al. found that MCL transection increased in situ ACL force by approximately 2.5 times when cadaveric knees were subjected to valgus torques\textsuperscript{65}. However, these studies were not performed under physiologic conditions and could not account for any biological significance these structures may have on overall joint
function after a period of healing. Although these studies have provided valuable insight, in vivo loading of the ACL and influences of surrounding structures have yet to be quantified.

Our research strategy employs a novel approach to overcome these barriers in measuring ACL force by using motion data recorded from the previously established sheep model and then using robotic technology to reproduce in vivo motion in the laboratory and determine native ACL forces throughout multiple physiologic loading paths and injury conditions.

**Robotic Testing**

Robotic technologies in conjunction with universal force sensors have offered researchers a testing platform which simulates motion paths while measuring the forces acting in the knee$^{32,48,67-71}$ (Figure 2.3.). Researchers can accurately and precisely control motions and test individual specimens in various knee conditions. To date, nearly all of the studies using robotic methods have simulated current clinical exams or applied forces/torques at specific flexion angles along a predetermined zero load path$^{32,67,68}$.

Popular clinical exams include the anterior drawer test, the Lachman’s test, and the pivot shift test. The anterior drawer test is conducted by positioning the knee at 90° of flexion and applying an anteriorly directed force to the tibia. This technique is applied to both the knee suspected of injury and the uninjured contralateral knee so that the resulting laxities may be compared$^{72}$. Similarly, the Lachman’s test is conducted by positioning the knee at 20-30° of flexion and following the same procedure for applying anterior tibial force both injured and contralateral knees as stated above$^{73,74}$. The pivot shift test is conducted by positioning the knee at full extension and applying an abduction (valgus) moment to the tibia, causing an internal rotation and anterior subluxtion of the tibia$^{73}$. The knee is then passively flexed with sustained
force being applied to the lateral side of the knee\textsuperscript{73,75}, producing a sudden external tibial rotation near 30° of flexion in the presence of excessive knee instability\textsuperscript{75}. One strategy of assessing ACL function is to simulate highly controlled versions of these clinical exams with prescribed knee translations and rotations\textsuperscript{1,32,68}. However, while essential for diagnosis, clinical exams are subjective assessments of passive joint laxity with little relevance to physiologic demands. These tests are performed at fixed flexion angles and only account for limited DOFs\textsuperscript{1,48,68,76-78}, failing to provide adequate insight into the complex role of the ligament during ADLs. Therefore, these tests provide a very limited evaluation of surgical success and are even more limited in quantifying in vivo demands.

Conversely, robotic technology allows researchers to simulate ADLs, assessing function in all 6 DOFs\textsuperscript{32,67-70} (Figure 2.3.). This capability is essential to examine the native function of the ACL during physiologic motion and to evaluate the ability of reconstruction methods to restore knee function. Ideally, these studies would be conducted within the human knee in vivo. However, this type of in vivo sensing in a human is limited by its invasive nature and would likely alter the dynamics of the knee joint and the ligament itself. Therefore, the control and accessibility granted using robotics offer an attractive alternative to in vivo sensing.
Figure 2.3. Schematic of each anatomical DOF as the tibia moves about the femur. While the knee is often simplified in computational models to only allow flexion and extension rotations, the remaining 5 DOFs play important roles in knee function and biomechanics.

The Sheep Model

While there are also in vitro limitations associated with testing cadaveric specimens, including specimen variability, age, quality, cost, and availability, these may be avoided by utilizing an animal model. More importantly, animal models allow for linkages to be established between in vivo and in vitro experimentation. For this reason, several large animal models with similar joint size and anatomy have already been developed to serve as biomechanical surrogates to the human knee\textsuperscript{79} (Figure 2.4.). Of these, the sheep model has been the most extensively characterized as a surrogate within our lab, and has demonstrated the greatest biomechanical similarities with respect to anatomy and simulated dynamics\textsuperscript{36}. With the added benefits of docility
and widespread availability, the sheep model has emerged as the most attractive surrogate for this research. Used in conjunction with robotics, the ovine (sheep) stifle (knee) joint will serve as a valuable development and screening tool for new and novel orthopaedic treatments. With future investigations focusing on translating to a cadaveric and/or clinical model, these initial surrogate findings will serve as the basis for future standards of patient care.

Figure 2.4. Comparison of human knee to sheep stifle joint.
Chapter 3

Effects of gait inclination on ACL and total knee forces during simulated in vivo motion in the sheep model

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Abstract

Purpose: Our current understanding of anterior cruciate ligament (ACL) function is based on simulations of clinical examinations and on the application of non-physiologic load and motion patterns. These methodologies likely provide little information on ACL function during activities of daily living (ADLs). More relevant metrics might include the strains and loadings of the ligament during active motions. However, due to the difficulties associated with in vivo sensing, researchers have yet to directly measure these physiologic demands on the ACL. Furthermore, it is unknown the extent to which these demands are altered due to changes in activity. This study aimed to quantify the effects of activity level on knee kinematics and corresponding joint and ligament loading profiles during simulated in vivo motions. Methods: Seven female Suffolk sheep were walked twice weekly on a treadmill during level (0°), inclined (+6°), and declined (-
6°) gait conditions for a total of 12 weeks. Electromagnetic (EM) tracking sensors were then surgically implanted onto the left distal femur and the left proximal tibia and in vivo motions were recorded post-implantation for all activities. Following sacrifice, each set of 6 degree-of-freedom (6 DOF) motions were applied to their respective knee joints using a serial robot instrumented with a multi-axis load cell. In vitro simulations were repeated to measure a) total knee loads, b) contact pressure maps, and c) ACL-only loads. **Results:** For each of the above response measures, declining gait led to: *Kinematics* – increased posterior translation during swing phase and decreased flexion at hoof-strike; *Joint Loads* – no significant differences; *Pressure Maps* – decreased medial contact at push-off; *ACL Loads* – decreased tension at hoof-strike and increased tension at push-off. Results of the this study are the first to relate ADLs to resulting knee biomechanics and ACL function and provide preliminary data for defining design requirements for future ACL reconstruction techniques.

**Introduction**

Injury to the anterior cruciate ligament (ACL) of the knee is a traumatic event which debilitates patients in both the short- and long-term. ACL injuries are common, particularly during athletic activities. Up to 250,000 ACL injuries are estimated to occur in the United States annually, accounting for approximately 8% of all soft tissue injuries, and significantly affecting both male and female athletes. However, female athletes are up to 10 times more likely than male athletes to experience injury per athletic exposure.

The ACL is the primary restraint to anterior tibial translation and is a secondary restraint to varus-valgus and internal-external rotations. Therefore, the loss of this structure causes patients to experience abnormal knee kinematics, which can lead to joint morbidity and pain.
As a result, surgical reconstruction of the ligament has become the standard for treatment, with the goal of restoring the anatomy and function of the native ligament. Still, studies show that while reconstruction does restore antero-posterior translation early after surgery, antero-posterior laxity increases with time. These altered kinematics can shift cartilage wear patterns to locations not accustomed to the loads, potentially leading to joint degeneration and osteoarthritis (OA). One study found that there was no decrease in the rate of early onset OA between patients who had reconstructions and patients who were treated conservatively. Recent studies of long-term outcomes for repairs indicate that between 50-85% of patients experienced early symptoms of OA within 11-20 years of surgery.

The underlying reason for this joint degeneration can be attributed to our inability to replicate normal ligament function, which is linked to our limited understanding of the complex role of the ACL during activities of daily living (ADLs). To improve surgical outcome and long-term patient prognosis after repair, it is critical to develop functionally based design criteria by which we can benchmark future repair techniques.

According to the principles of functional tissue engineering, a ligament’s function may be defined as its in vivo loading characteristics. While this information remains impossible to measure directly in human patients in vivo, in vitro testing can serve as an alternative as long as applied kinematics match in vivo ADLs and loads are measured within the same limb from which the kinematics were derived. Therefore, combining robotic simulation with an in vivo animal model provides a powerful tool for investigating ACL functionality. Utilizing robotics allows open access to the joint to measure contact forces and 6 DOF ligament loads throughout physiologic motion paths. Utilizing an animal model allows specimen-specific kinematics to be
collected and applied to the same tissue, overcoming several limitations of cadaveric testing, including specimen quality and kinematic mis-matches.

Given these advantages, this study relied on robotics in combination with the sheep knee model for physiologic approximations of in vivo loading which may serve as baseline design criteria for future ACL repairs and reconstructions.

**Methods**

*Experimental Design*

Seven (7) skeletally mature, female Suffolk sheep (age: 2-6 yrs.; weight: 100-200 lbs or 45-90 kg.) took part in this IACUC approved study, with five advancing all the way through to robotic testing due to an uncooperative animal and surgical complications. Animals were skeletally mature with closed epiphyses and we screened for normal gait prior to inclusion. Based on the inter- and intra- animal consistency observed in our previous studies\(^8\), level, inclined, and declined gait were chosen as the three ADLs to be investigated. These ADLs have also been shown by our lab to result in unique biomechanical responses as indicated by vertical ground reaction forces (VGRFs) and arthroscopically implantable force probe (AIFP) response\(^8\). All sheep were walked twice weekly at 1m/s during level (0°), inclined (+6°), and declined (-6°) gait conditions on an instrumented treadmill (Kistler Gaitway) to collect VGRFs for each ADL over a period of 12 weeks. While animals were fully trained in the walking protocol and achieving consistent VGRFs within 3 weeks, the 12 week time point was selected so that this cohort could be used as a control group for future studies investigating healing response. After 12 weeks, electromagnetic (EM) trackers were rigidly implanted on the proximal tibia and distal femur. Post-operative VGRFs and gait kinematics were collected at 2, 6, and 9 days to establish in vivo motion profiles for each ADL. Following sacrifice, these motions were reproduced on each respective
specimen using a 6 DOF serial robot. A 6-axis load cell recorded forces and torques of the total knee joint throughout the range of each motion while thin-film sensors (Tekscan) captured the joint pressure map between each condyle and the tibial plateau. All structures of the knee were then removed, including the bony interactions, leaving only the ACL to transmit loads across the joint. Each motion was performed again to record the ACL loads specific to each ADL.

Motion Tracker Implantation

After 12 weeks of treadmill training, animals underwent surgery to implant EM tracking sensors (Polhemus; Liberty) onto the anteromedial side of the distal femur and proximal tibia of the left hind limb. Surgeons (Dr. Marc Galloway, Dr. Sam Harms, or Dr. Michal Taylor) accessed the knee via a medial incision. The tibial tracker was placed inferior to the joint line onto the medial aspect of the tibial tubercle. The femoral tracker was placed on the medial epicondyle by detaching the vastus medialis oblique (VMO) from the patella and reflecting it posteriorly. Trackers with lead wires were passed sub-cutaneously from the flank to the knee, leaving the tracker connections to exit the side of the animal. Trackers were fixed using two self-tapping orthopaedic screws per tracker, making sure that they passed through both cortices. The VMO was then reattached over the top of the femoral tracker before closing with suture. Tracker connections were secured on top the animal to prevent damage from activity or chewing.

In Vivo Data Collection

Post-implantation, animals were allowed to ambulate freely within their pens. Kinematic data were collected at 2, 6, and 9 days post-surgery along with VGRFs. Previous data had shown that VGRFs return to >80% of normal values by 9 days\textsuperscript{82}. Pilot studies with the Liberty system
showed higher accuracy when using the larger 4” EM source option placed at the data collection distance of between 35 and 50 cm. During data collection, all metal objects were eliminated from the field with the exception of the treadmill itself. To ensure that the animal could complete each ADL, level gait was measured first, followed by inclined gait, followed by declined gait, which appeared to be the most difficult to complete for post-implantation animals. Once the animal reached the test speed of 1 m/s, kinematic data recording was initiated at a rate of 240Hz and an independent binary signal was introduced to both the VGRF and kinematic data to later sync the outputs. Translations and direction cosigns of both trackers were recorded for a minimum of 5 consecutive gait cycles for each ADL. Data was chosen from the collection date with VGRFs matching most closely with pre-implant data. A custom MATLAB program then transformed this data into the position and orientation of the tibial tracker with respect to the femoral tracker. This relative positioning technique was used to help mitigate any error introduced by metal in the field. Kinematic data was normalized by gait cycle percentage to establish the specimen-specific average joint motion cycle for each sheep during each ADL.

Robotic Simulation

A high accuracy (± 0.06 mm reliability) serial robot (KR210; Kuka Robotics Corp., Clinton Township, MI) was used to recreate motions in vitro. Animals were sacrificed after day 9 of data collection and limbs were harvested and frozen at -20° C until the day before testing. Limbs were dissected free of all soft tissues leaving only the 4 knee ligaments, menisci, and joint capsule. Using anatomical landmarks according to Grood & Suntay\textsuperscript{84}, joint coordinate systems were established for both the tibia and femur. Care was taken to be sure that the orthogonality driven by these landmarks was sufficient so that the matrix describing each coordinate system had a
determinant of at least 0.997, allowing for accurate kinematic calculations. Orthogonality adjustments were always made by adjusting the mark corresponding with the origin of the lateral collateral ligament (LCL) to a more proximal and/or posterior location. The tibial center point (TCP) was considered the most proximal point of the medial tibial spine while the femoral center point was considered the anterior-most point of the femoral notch where the patella-femoral cartilage begins. The distal tibia was then removed and the tibia was potted into a custom fixture used to align it with the robotic end effector. A mold was placed around the long axis of the femur to create a key with which to rigidly secure the femur into the table fixture during testing. All tests were performed at room temperature with the joint wrapped in saline soaked gauze to prevent soft tissue dehydration.

Using a coordinate measurement machine (CMM, Faro Digitizer F04L2, FARO Technologies Inc., Lake Mary, FL), the coordinate systems of each of the bones were established relative the respective tracker coordinate system. With this information, anatomic motion of the tibia with respect to the femur could be derived from the in vivo tracker data in accordance with the commonly used knee joint coordinate system\textsuperscript{84}. While the resulting anatomic motions were mostly in line with expectations, the sign of the compression/distraction curve was flipped to more closely represent increased compression during stance and increased distraction during swing. Final kinematics are reported as one cycle of anatomic motions with translations relative to hoof strike and rotations as absolute values. In vivo anatomic kinematics were then transformed into the robotic coordinate system following the roll-pitch-yaw rotation convention. Using a Robotic Sensor Interface (RSI) package, the desired motion path was sent to the robot in incremental pieces rather than absolute positions. Therefore, an iterative approach was used in developing the each motion path to assure that incremental errors in position did not accumulate over the entire motion.
Mid-stance was chosen as the start and end points of the gait cycle to minimize positional error associated with overcoming high impulses as the robot starts and stops its motion. Therefore, all data reported have been shifted to begin reporting at hoof strike.

With the tibia rigidly fixed to the robotic end effector and the femur secured to the test table, the CMM was used to position each of the specimens into their respective mid-stance starting orientations within ±1° of the calculated value. Mid-stance translational positions were calculated but absolute positions were not used due to the non-physiologic loading experienced by the knee when attempting to impose these values. Therefore, while relative translational motions were still applied according to in vivo measurements, the absolute translational starting position was determined by applying load until a calculated amount was achieved in each DOF according to the inverse dynamics using measured VGRF and kinematic data. Because ACL loading is the primary interest of this study, an additional anterior starting point was also established by moving the tibia anteriorly and superiorly from this point until posterior force was eliminated but compression force remained as calculated. This created a second starting point from which ADLs could pose a greater challenge to the ACL throughout the applied motion. Tibial motions for each of the 3 ADLs were then cycled from each of their respective starting points for a total of 6 simulations per measurement (intact load, pressure maps, ACL load, and gravity loads). For all simulations, the TCP was defined as the robotic tool while the starting point of the TCP was defined as the base about which motion occurred. Incremental motion commands were fed to the robot every 12 ms (a rate of 83.33 Hz). To maintain kinematic resolution, motions were applied at approximately 1/5th of in vivo speeds. Each simulation repeated its normalized kinematics for a total of 10 cycles to minimize viscoelastic effects.
**Verification of Kinematic Accuracy**

To independently verify that in vivo motions could be reproduced using these described methods, a small study was performed to assess the associated kinematic error. Three cadaveric sheep limbs fitted with EM trackers were attached to a polymeric fixture placed over the running treadmill to simulate the in vivo scenario (Figure 3.1). The limbs were secured at five different flexion angles, and the positions were recorded using both the EM tracking system and the CMM. The anatomic knee positions were then determined, using anatomic landmarks as described above, and the limb was attached to the robot. Following identical procedures for robotic installation and motion development, specimens were aligned to the rotations corresponding to each set of orientations collected on the polymeric fixture. Translational forces were then zeroed to reproduce conditions during collection of the original data. At each of the five reproduced flexion angles, the CMM was again used to determine the 6 DOF anatomic joint position and point-to-point distances between selected bony landmarks on the tibia and femur. These positions and point-to-point distances were compared between the simulated in vivo condition (polymeric fixture over the treadmill) and the in vitro robotic condition.
Figure 3.1. Assessing Kinematics Accuracy. Both EM data and CMM data were recorded at 5 different flexion angles within the testing volume of in vivo data recording. Positions were later recreated on the robot and robotic CMM data were compared to measurements made here.

In Vitro Data Collection

During the applied motion, force and torques applied to the specimen were recorded using a six-axis load cell (Theta Model FT5498; ATI Industrial Automation, Apex, NC) aligned and rigidly secured to the robotic end effector. The tibial fixture was secured directly to the face of the load cell. All load data was reported with respect to the tibial coordinate system at the TCP using a mathematical transformation. Upon completing each increment of the motion command, the robot and load cell reported the calculated position along with measured loads. Thus, load data was also collected every 12 ms (or at 83.33 Hz). In vitro testing began by applying each of the 6 simulations (each of the 3 ADLs at the anterior position followed by the 3 ADLs at the posteriorly
loaded position) on the intact specimen knee. To minimize viscoelastic effects of applied start loads, the order of applied ADLs began with declined and ended with inclined, as the inclined condition almost always carried the most load during mid stance. 6 DOF forces and torques were recorded throughout the gait cycle for each ADL in this intact condition. Intact knee loads were reported as percent body weight (%BW) for each animal.

Joint contact pressure maps were then measured by sectioning out the medial collateral ligament (MCL), lateral collateral ligament (LCL), posterior cruciate ligament (PCL), and any remaining joint capsule tissue. This made room for the pressure sensor (Tekscan; 4000 model; South Boston, MA) while leaving the ACL and 2 menisci intact. Both bifurcations of the thin film sensor were placed between the menisci and the femoral condyles on both the medial and lateral sides. This placement was chosen over direct contact with the tibial plateau to better quantify the contributions of the menisci to the overall loading patterns within the knee. Therefore, direct measurements of tibial loading were not collected. Rather, we observed the interaction between the femoral condyles and the proximal surfaces of the menisci. Sensor tabs were secured onto the tibia so that the sensor was attached as it moved about the femur. Sensors were calibrated with the load cell after 5 minutes of static loading at ¾ of the maximum compression observed during the prior simulations. Each of the 6 simulations were then repeated and pressure data was recorded using I-Scan software at a rate of 4 Hz for the duration of each test.

To measure ACL loading, both menisci were removed along with the pressure sensors and each of the femoral condyles were sawed off to prevent compressive loading. When removing the lateral condyle, care was taken not to disturb the attachment of the ACL origin into its medial aspect. Using this strategy, only the ACL remained to transmit any load. Simulations were again repeated for this ACL-only condition while the load cell recorded 6 DOF forces and torques.
Finally, the ACL was removed and simulations were repeated recording only the effects of gravity on the tibia and its fixture. These values were subtracted from all previous load measurements.

**Data Analysis**

All kinematic and load cell data were averaged over a single cycle and average and SEM values are reported here. Effects of ADLs were then evaluated using one-way ANOVA testing with three levels of the test factor (surface inclination) to compare means of each ADL condition with respect to average value, maximum and minimum values, and range of each DOF. Data were then broken up into stance and swing phases and ANOVA tests were re-applied to each of these data sub-sets. All statistically significant response measures were found to be normally distributed and homoscedastic. Responses that were not normal or homoscedastic were typically widely varying within the sample, leading to very low power values and higher probabilities of type II error.

**Results**

**VGRFs**

Comparisons between ADLs prior to implanting the EM trackers showed significant differences in average VGRF value between each grade condition ($p< 0.05$) and peak values of both the left and right hind limbs when comparing declined to inclined conditions ($p< 0.05$, Figure 1A). Post-implantation data showed similar trends, with significant differences in average VGRF and left (implanted) hind limb peak force ($p< 0.05$). However, peak values of the uninjured right limb VGRFs began to converge to a common point and were no longer statistically different
Comparisons between pre and post-implantation VGRFs show that while average VGRFs for declined and inclined conditions were statistically different (p< 0.05) along with peak left (implanted) VGRFs for the declined condition only (p< 0.01), relative trends between ADLs remained the same.

Figure 3.2. A. Average data for pre-implantation VGRFs for each ADL normalized to a gait cycle. Cycles begin and end with hoof strike and VGRFs are symmetric between right and left limbs. B. Post-implantation VGRF data demonstrating altered gait patterns due to limping while maintaining the relative differences between ADLs. (SEM bars).

Kinematics

In vivo kinematics were different between all ADLs, with the greatest statistical differences in anterior/posterior translation and flexion/extension rotations. Directionally, declining gait led to increases in posterior translation, medial translation, and distraction throughout gait; decreases in external rotation and increases in flexion during push off; and increases in external rotation and decreases in flexion during hoof strike (Figure 3.3.). Statistically, average translations during
swing phase were more posterior during declined gait (p= 0.055), and peak posterior translations were higher (p< 0.05). Additionally, the range of flexion during stance was larger during declined gait (p< 0.05). At hoof strike, extension was observed to be higher during declined gait, but this finding was not statistically significant due to high variation, non-normality, and low statistical power (as low as 6%).

**Figure 3.3.** Effects of ADL on Kinematics. Average in vivo knee kinematics with SEM bars for all ADLs: level (0°), inclined (+6°), and declined (-6°) gait. The gait cycle begins with hoof strike. Swing phase begins at approximately 62% of the gait cycle. Clear patterns are observable as gait is declined.

**Kinematic Accuracy**

Compared to the anatomic positions calculated using EM tracker data recorded in the treadmill environment, in vitro robotic CMM data showed low error for rotational values,
demonstrating that positions were accurately reproduced in internal/external (0.95 ± 0.59°), flexion/extension (0.50 ± 0.34°), and ad/abduction (1.76 ± 0.83°) degrees of freedom. Rotational error increased with flexion angle, with a maximum calculated value of 2.95° ad/abduction error at 80° flexion for one specimen. Translational errors were much greater, with anterior/posterior (34 ± 12 mm), medial/lateral (5 ± 3 mm), and compress/distraction (39 ± 4 mm) DOFs all greatly exceeding physiologically possible values. This may be attributed to the metal treadmill’s interference with the EM system. However, by using only the zero load condition to position the translations of the specimens, point-to-point distances measured via CMM were reproduced within a millimeter when comparing tibial tubercle to femoral head (0.43 ± 0.28 mm), MCL origin to insertion (0.56 ± 0.44 mm), and tibial tubercle to proximal femoral notch (0.42 ± 0.24 mm) measured distances. One specimen had a particularly orthogonal joint coordinate system prior to the landmarking process (determinant of 0.9943), which allowed for CMM data to be used to calculate anatomic position in the polymeric fixture during original data collection. Compared to the reproduced position of this specimen in the robot fixture, the highest translational difference ranged from only 0.74 mm at 80° flexion to 6.38 mm at 40° flexion in the anterior/posterior direction. However, this orthogonality was still lower than acceptable for kinematic calculations in this study, which makes this error calculation less accurate. This may explain why the anatomic translational differences calculated via CMM were higher than point-to-point deviations.

**Intact Knee Loads**

Comparisons between loads recorded during simulations initiated from the original, posteriorly loaded starting point and loads recorded during those initiated from the anterior starting point showed minimal differences aside from this anterior/posterior force at midstance. Therefore,
because simulations initiated from the anterior stating point posed a greater challenge to the ACL, only simulations initiated from the anterior starting point will be presented here.

Intact knee forces and torques showed modest differences between ADLs, especially at hoof strike and push off. Declining gait led to increased posterior forces and corresponding extension torques during hoof strike. At push off, declining gait led to decreased posterior force and compression as well as decreased internal and extension torques. In addition, declining gait let to a transition from medial force and abduction torque during stance to more lateral force and adduction torque (Figure 3.4.). However, none of these relationships were determined to be statistically significant. No differences were observed during swing.

**Figure 3.4.** Effects of ADL on Intact Knee Loads. Average intact knee loads during in vitro simulation with SEM bars for all ADLs: level (0°), inclined (+6°), and declined (-6°) gait. The gait cycle begins with hoof strike. Swing phase begins at approximately 62% of the gait cycle.

Moderate patterns are observable as gait is declined.
Pressure Maps

While each specimen produced unique pressure maps corresponding to the individual geometries, patterns did emerge when comparing ADLs across each stage of the gait cycle. The most prominent observation was the distinction between ADLs during push-off. Inclined gait produced high levels of pressure in the medial compartment of the knee compared to the lateral compartment (Figure 3.5.), with most of the medial compartment pressure transmitted from the condyle to unprotected tibial cartilage. As gait transitions to level, medial pressure drops significantly while lateral pressure remains about the same. Medial pressure continues to be transmitted from condyle to exposed tibial cartilage. At declined gait, there is a drastic reduction in pressure within both compartments. Overall, compared to level gait, inclined gait increases pressure only in the medial compartment while declined gait decreases pressure across both compartments.
Figure 3.5. Effects of ADL on Pressure Maps at Push-Off. Representative in vitro knee joint pressure maps. Anterior is oriented toward the top of each plot and posterior is oriented toward the bottom. Push-off occurs at approximately 50% of the gait cycle.

ACL Loads

Reported results were recorded during kinematic simulations initiated from the anterior version of the starting position, which posed a greater challenge to the ACL and resulted in higher resolution between ADLs. Declining gait led to lower anterior, medial, and distraction forces at hoof strike coupled with transitions from flexion to extension and abduction to adduction torques within the ACL (Figure 3.6). During push off, declining gait led to slightly higher anterior,
medial, flexion, and abduction forces and torques. Maximum distraction force within the ACL during stance was higher for inclined gait compared to declined gait, though statistical power was too low (between 20 and 45%) to establish significance. No differences were observed during swing.

Figure 3.6. Effects of ADL on ACL Loads. Average ACL loads during in vitro simulation with SEM bars for all ADLs: level (0°), inclined (+6°), and declined (-6°) gait. The gait cycle begins with hoof strike. Swing phase begins at approximately 62% of the gait cycle. Moderate patterns are observable as gait is declined.

Discussion
This study successfully examined the relationships between kinematics, total knee loads, contact pressures, and ligament loads for multiple ADLs. As expected, results demonstrated that
declining gait decreases flexion of the knee at hoof strike and increases flexion during push-off. These differences also appear to be the driving mechanisms behind kinematic differences in the anterior/posterior and compression/distraction DOFs. Compared with hoof strike values, declining gait resulted in more posterior and less compressive translations during push-off. This can be explained by the location of the femoral coordinate system at various stages of flexion. Anatomic kinematics are reported with respect to the femoral coordinate system, which resides in the femoral notch. Due to the elongated geometry of the femoral condyles, as the knee flexes, this femoral coordinate system moves superiorly with respect to the tibial coordinate system. By the time the knee reaches 90°, the A/P axis of the femur is aligned with the C/D axis of the tibia. This means that a more “posterior” position of the tibia with respect to the femur will be achieved even during passive knee flexion. Therefore, any increase in posterior translation during periods of increased flexion should not be considered to have the same biomechanical implication as a pure translation without a coupled rotation. Declined kinematics also showed greater medial translations compared to hoof strike and greater internal rotation during push off. However, only sagittal differences were statistically significant.

Differences in sagittal kinematics directly translated to differences in knee loading patterns. However, while declining gait showed trends of more posterior tibial translation during push-off compared to hoof strike, intact knee loads during push-off were actually less posterior compared to hoof strike, and could even cross briefly into anterior loading. Similarly, inclined gait showed trends of more anterior tibial translations at push-off but more posterior forces compared to hoof strike. These seemingly contradictory events may be explained by the change in coordinate systems between kinematic and dynamic reporting. As described above, kinematics are reported as the position of the tibia with respect to the femur. However, dynamics are reported in the
tibial coordinate system. Therefore, at high flexion angles a “posterior” translation coupled with high flexion may not correspond to any change in loading.

Another contributing factor is the presence of joint laxity wherein it is possible a pure translation may not cause loading. This is especially applicable during swing phase. Conversely, high levels of bony interference could amplify load changes for even the smallest translations. For example, during inclined push off the knee experiences greater compressive translations compared to hoof strike. Combined with the coordinate system change from kinematic to dynamic responses, posterior loading at push off is expected to increase even though kinematics are more anterior. This theory is supported by joint pressure maps which show significant decreases in tibio-femoral contact at push off as gait is declined. Previous studies in our lab have shown that the bony interaction accounts for the vast majority of intact knee loads, followed by the medial meniscus\(^7\). This would explain why pressure map data shows the medial compartment as the most affected by ADL.

Differences in ACL loading patterns were similar to differences in total knee loading. The inclined ADL produced greater anterior, medial, distraction, and abduction loads at hoof strike while the declined ADL produced greater anterior and abduction loads at push off. However, the highest magnitudes of ACL loading were during swing phase. These results indicate that, in our in vitro simulations, ACL loading is measured with the greatest resolution during less-compressed knee states. While we may infer that ACL function is more critical during load bearing activity to prevent injury, previous studies have shown that increased compression while bearing load does not lead to any increase in ACL strain. One study compared ACL strain during active flexion with no resistance to ACL strain during squatting with body weight and found no difference\(^8\). A more recent study showed that while the addition of external compression to the knee does not reduce
strain in the ACL during normal flexion and extension, compression did shield the ACL from additional strain when resistance was introduced\textsuperscript{86}. This type of compressive shielding has also been demonstrated in previous studies in our lab during both cadaveric and sheep knee testing\textsuperscript{70}. It may be explained by compression bringing the origin and the insertion of the ligament closer together, thereby reducing the tensile load transmitted through the structure at these times.

Another possible explanation for low ACL loading during stance is artefact caused by “expansion” of the remaining bones after removal of the articulation to achieve the ACL-isolated condition during in vitro simulations. Without the femoral condyles present to properly fill the joint space between femoral origin and tibial insertion points, the remaining uncompressed bone may have been be free to expand further into the joint space, bringing the two ends of the ligament closer together and reducing overall tension on the ACL. While this would also affect loading during swing phase, this phenomenon coupled with compressive shielding could explain why minimal load was observed in the ACL during phases of each ADL when compressive load was highest.

These relatively low ACL loads may be a limitation of using the sheep model for robotic testing, as their knees have been shown to be tighter than the human knee joint\textsuperscript{36}. This could accentuate the effects of compressive shielding and expansion artefact on measured isolated ligament loads. Previous studies in our lab have shown that while the human ACL is the third highest contributor to total intact knee loads (behind bony interaction and medial meniscus), the sheep ACL, measured by the same methods presented here, remains a relatively small contributor\textsuperscript{70}. However, while ACL loading may be low, current literature still supports the use of sheep as surrogate for ACL biomechanics, as it is critical to overall knee function.\textsuperscript{87,88}
Several other limitations existed within this study. First, a relatively low sample size (N=5) and high variation led to low statistical power, leaving us unable to statistically detect differences between ADLs during in vitro simulation. Power analyses determined that with the levels of variation observed in this study, a sample size of 10 or more would be more appropriate. It is also possible that some of the variation was due to kinematic error driven by EM tracking system interference, which could have influenced the ADL motions. While metal in the measurement field was kept to a minimum, the treadmill itself was enough to introduce error into measurements during pilot studies. To mitigate these effects, only relative tracker positions were used, so that this error would cancel out assuming both trackers were equally affected by the interference. Still, residual error from this process and error from compounded measurements and calculations had the potential to stack up and significantly impact motions. However, results of the kinematic accuracy testing showed that while kinematics are most prone to error in ad/abduction at high flexion angles and anterior/posterior error at low flexion angles, these errors did not notably affect the resulting loads measured in the study, and can be considered negligible. Finally, while we are confident in our measurement equipment and replication methodologies, the post-operative in vivo kinematics themselves were subject to alteration in response to implantation of the two trackers onto the limb. While animals did demonstrate a visible limp during post-implantation gait, comparisons of pre-op to post-op VGRFs showed no relative differences between ADLs.

Despite these limitations, our methodology has proved effective in demonstrating differences in functional ACL demand between varying ADLs. Results suggest that, while varying degrees of inclination produces significant kinematic differences throughout the gait cycle in the sagittal plane, differences in ACL loading are less observable due to compressive shielding during stages of ADLs when higher proportions of body weight are applied to the joint. Thus, inclined
gait showed the greatest challenge to the ACL during hoof strike (0% gait), while declined gait showed the greatest challenge to the ACL occurs during push-off (50% gait). This finding challenges the notion that ACL loading is strongly linked to ground reaction force, a common input to computational ACL models, which has implications for designing future ACL replacement techniques. According to these results, the greatest potential for activity-driven functional differentiation lies within the transition periods between unloaded and loaded knee states with the largest ACL challenges occurring at instances of increased flexion angle. This is consistent with many theories surrounding ACL injury prevention.

This study contributes to a more thorough understanding of knee biomechanics and testing strategies as they relate to ACL function, and provides preliminary data for defining design requirements for future ACL reconstruction techniques. Physiological ADLs were successfully applied to each knee from which they were derived, and resulting joint dynamics can be clearly described and explained. This robotic testing platform will continue to be utilized to examine many more factors governing ACL function and repair, with the goal of informing better ACL reconstructions to slow or stop the early onset of OA.

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Chapter 4

Impacts of robotic compliance and bone bending on simulated in vivo knee kinematics

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Abstract

Purpose: Robotic testing offers researchers the opportunity to access the structures of the knee joint during activities of daily living to quantify native tissue loads. These loads may then be translated into design requirements for future treatments and procedures to combat the early onset of knee degeneration following an injury. However, high knee loads during testing have the potential to deflect a robotic end effector, causing inaccuracies in the applied kinematics. Furthermore, bone bending could also induce kinematic change. This study aimed to quantify the effects of robotic compliance and bone bending on the accuracy of simulated in vivo kinematics in a KUKA KRC210 serial robotic system. Methods: Six (6) human cadaver knees were subjected to cyclic human gait motion while 6 DOF forces and torques were recorded at the joint. A Vicon T-Series camera system was used to independently record the applied kinematics. Results: The more flexed periods of gait produced the greatest kinematic deviations,
corresponding to instances of low joint loading. However, in many cases, low tibial and femoral orthogonality calculated from joint coordinate system landmarks made accurate calculation of anatomical positions mathematically impossible. While results of this study suggests that high physiologic loading does not lead to deviation from target and kinematics, further testing is necessary to confirm.

Introduction

Current treatment and reconstruction methods of native knee tissues fail to prevent the early onset of osteoarthritis (OA) and knee joint degeneration. To improve patient outcomes, we must better quantify the functional demands placed on these knee structures during activities of daily living (ADLs). However, functional loadings remain impossible to measure directly in-vivo. To overcome this limitation, many researchers have developed robotic testing methodologies to simulate in vivo motion in the laboratory, allowing greater access to measure forces and torques within the joint. These measured tissue loads offer realistic alternatives to true in vivo sensing, provided that the in vitro system accurately represents the dynamic conditions present during in vivo ADLs. While several studies have demonstrated the ability to accurately collect kinematic data during ADLs, little data exists supporting the ability to accurately reproduce these kinematics on human tissue specimens using robotics. While these inputs should be precise in an ideal system, it is possible that the high knee loads generated during ADLs may alter the path of a robotic end effector or cause bone bending during testing. Either of these events could alter the applied kinematics at the joint. To better understand this phenomenon and to assess deviation within our own laboratory, this study aims to 1) apply previously recorded in vivo kinematics to human knees in vitro, 2) independently measure the simulated kinematics and applied loads, 3) quantify the effects of robotic compliance and/or bone bending.
Accurate reproduction of in vivo kinematics using the robotic system relies on the principle that the system itself is much stiffer than the test object (the knee joint). While this is an intuitive assumption, the actual compliance of the robot and bony fixation interfaces in our lab have yet to be quantified. Using an external motion capture system, reflective marker points on the robotic end effector, proximal tibia, and distal and proximal femur were tracked during human gait simulations. Human gait kinematics were previously recorded by Lafontune et al. using transdermal bone pins\textsuperscript{89}. Using a coordinate measurement machine (CMM), these markers were translated into the joint’s anatomical reference frame to provide kinematics measured independently from the robotic software. By comparing the kinematics reported by our robot to the actual kinematics measured by the Vicon system, we may calculate any divergence in desired motion caused by either robotic compliance or specimen bone bending to arrive at the overall compliance of the test setup. This information will be used to assess the general kinematic accuracy of our methodology to apply physiologic motions and will be invaluable as we proceed with this robotic testing platform, investigating more aggressive ADLs which produce higher loads across the joint.

**Methods**

*Robotic Manipulation*

Six (6) cadaveric specimens (no pairs) were used in the study. Three specimens were procured from the University of Cincinnati Body Donor program and were females in their 80’s with an average body weight of approximately 120 ± 23 lbs. The other three were procured from a tissue bank (Anatomy Gifts Registry, Hanover, MD) and were males in their 50’s with an average body weight of 170 ± 43 lbs. All specimens were dissected free of all soft tissue leaving only the menisci and the cruciate and collateral ligaments with surrounding joint capsule. For each
specimen, the tibia was potted into a custom built adjustable fixture using PMMA bone cement which allowed its anatomical axes to be aligned with the robotic end effector coordinate frame. A PMMA mold was also applied to the femur as close to the joint as possible which allowed it to be rigidly bolted into a fixture on a stationary testing platform.

A six-camera, infra-red motion tracking system externally recorded specimen motion (Vicon; T-Series). A total of 16 reflective markers (10mm in diameter) were attached to each test specimen using a combination of double sided tape and super glue. Sets of 4 markers each were attached to 1) the base of the robotic end effector near the axes, 2) near the joint line onto the cortical bone of the proximal tibia, 3) near the joint line at the distal femur, and 4) on the other side of the femoral fixture at the proximal femur (Figure 4.1.). Six cameras were placed around the work space to record the motion of the reflective markers at a rate of 120 Hz. Camera calibrations revealed an average residual of 0.674 ± .09 mm.
Figure 4.1. Posterior view of a specimen with reflective markers attached. All other potentially reflective surfaces were covered with black matte paper.

Anatomical axes of the knee were established using the Grood/Suntay method\textsuperscript{84}, which used the origins and insertions of the collateral ligaments to define the medial/lateral axes of each bone and the medial tibial spine and femoral notch as the bony origins. Using a CMM, the tibial coordinate system was aligned with and rigidly fastened to the robotic end effector. The robot was then driven to a position minimizing the loads within the joint and the femur was secured to the table test fixture. The CMM was then used to register the current anatomic position of the knee along with the relative positions of each of the 16 reflective markers within the robotic coordinate system (Figure 4.2.) Markers were placed as close to the joint axes landmarks as possible to
prevent relative motion between them. This initial measurement was fed into a custom MATLAB program that calculated the corresponding robotic moves needed to align the knee to the starting orientation at mid-stance of gait. Once all joint angles were verified within 1 degree, translational loads were minimized to establish an initial start point for robotic manipulation. Compression was added to this starting point as needed to achieve a peak force between 2.0-2.5 times body weight\textsuperscript{90} when gait motion was applied.

![Markers on Specimen in Robot Coordinate System](image)

Figure 4.2. Reflective marker positions as measured in the robotic coordinate system using the CMM. Tibial and femoral center points are examples of bony landmarks used to define the tibial and femoral coordinate systems. Absolute distances from bony landmarks to each of the markers in the closest cluster were used to place landmarks within the marker coordinate system.
Using data recorded from Lafortune et al., a 6 DOF gait motion was digitized to be replicated by a KUKA KRC210, position accurate, serial robot with a 210kg rated payload (~2.5 x BW). Peak flexion during mid-stance was chosen as the starting and ending point for each gait cycle to be applied. All translations and rotation were to occur about this starting pose. Anatomic motion was transformed from its original joint coordinate system order of rotations to the necessary robotic inputs which use the customary roll-pitch-yaw notation. Gait kinematics were then applied at an 8 fold reduction of in vivo velocity for 10 cycles of preconditioning, followed by another 10 cycles of data collection. Specimens were kept hydrated via saline soaked gauze for the duration of the test. A multi-axis load cell (ATI; Theta Transducer) recorded forces and torques transmitted across the knee joint while the Vicon system recorded the positions of the reflective markers throughout testing.

Data Analysis

Forces and torques of each specimen were filtered using a Fourier transfer to eliminate high frequency noise and normalized to body weight before they were averaged into a single gait cycle. Marker data was labeled within the Vicon iQ software workspace and exported to spreadsheets. A custom MATLAB script cleaned the data by unifying trajectories for markers of the same label and eliminating dual instances caused by artifact. A moving average filter applied to each DOF of data extracted noise above 20 Hz. All 10 test cycles were then averaged together to establish overall marker trajectories for a gait cycle. Test cycles missing full or partial spans of data due to “lost” markers were eliminated from the overall calculation. Kinematic standard deviations in cycle trajectories averaged approximately 0.3mm.
Marker data was then used to generate anatomic kinematics at the knee joint itself. In an unloaded position, the robotic coordinate system and the tibial aspect of the joint coordinate system (JCS) are identical, aside from an offset of up to 30cm, and the femoral aspect of the JCS is stationary (Figure 4.3.). Anatomic kinematics are reported assuming the tibial and femoral bones are rigid and the offsets from each bone’s JCS to the known or stationary fixture positions are constant throughout testing. However, even with short distances from the joint to each of the fixtures, the bones may be able to flex with respect to the fixtures in high load conditions, causing deviation from the reported kinematics. Therefore, markers were clustered as close to the tibial and femoral JCS landmarks as possible to minimize relative motion between them, allowing the camera system to independently assess the position of the actual JCS during testing.
Figure 4.3. Robotic test setup including each of the coordinate systems referenced in this study:

A. Robotic coordinate system, B. Marker coordinate system, C. Femoral coordinate system,

D. Tibial coordinate system.

Trajectories were used to reconstruct actual positions of the tibial and femoral coordinate systems of the knee joint (Figure 4.3.C. and 4.3.D.) using CMM data to place joint landmarks within the marker reference frame (Figure 4.3.B.). All landmarks and marker sphere positions were recorded with respect to the robotic coordinate system during specimen set up (Figure 4.2
and Figure 4.3.A.). To place each landmark into the marker coordinate system, absolute distances from each of the 4 markers in the nearest cluster were calculated from the CMM data, and a system of equations was solved to place the landmark in the position which minimized the error compared to recalculated absolute distances within the marker coordinate system. This placement was iterated for every landmark at every frame of the gait cycle, resulting in a record of independently measured tibial and femoral positions. Coordinate transformations were used to calculate anatomic kinematics as the motion of the tibia about the femur. This data was then compared to the target kinematics.

Kinematic error was compared to the corresponding forces and torques recorded during the motion. For each specimen, correlation coefficients were computed between the error in each DOF and the forces and torques being applied at the corresponding time point. For a sample size of 6, the critical correlation coefficient value is 0.811 for an alpha value of 0.05. If, at any point during the simulation, the determinant of the tibial or femoral position matrix fell below 0.994, this data was excluded from the correlation coefficient calculations. As matrix determinants are measures of orthogonality, the further it is from 1 (perfectly orthogonal), the more numerical instabilities and inaccuracies are introduced into positional calculations. For reference, determinants of specimen coordinate systems in our current studies must be above 0.997 in unloaded conditions to ensure that target positions are accurately achieved within ± 1 degree.

Eliminated Data

While all specimens were verified to be orthogonal by the CMM during setup at a zero-load condition, orthogonal bony coordinate systems were unable to be derived from the recorded marker data in three of the six experiments. The calculated coordinate systems of these specimens
were never orthogonal enough to achieve numerical stability, preventing mathematically accurate calculations of their kinematics. The average femoral orthogonality of the remaining three specimens was calculated to be 0.998 ± 0.002, and average tibial orthogonality was calculated to be 0.989 ± 0.015.

Results

Using only orthogonal data across the gait cycles of each of the 3 numerically stable specimens, the average anterior/posterior kinematic error of the tibia with respect to the femur was calculated at 8.1 ± 13.6 mm posterior; medial/lateral error at 6.9 ± 7.8 mm medial; superior/inferior error at 6.3 ± 9.6 mm superior; internal/external error at 3.0 ± 6.9 ° external; flexion/extension error at 5.1 ± 5.0 ° flexed; and ad/abduction error at 6.8 ± 3.9 ° abducted compared to the input kinematics (Figure 4.4.). Highest deviations from the target kinematics occurred during the swing phase of gait, when knee loads were at their lowest (Figure 4.5.). Correlation coefficients of the same subgroup were largest for medial deviation and medial force (0.68), medial deviation and distractive force (-0.73), and medial deviation and flexion torque (0.73).

However, considering only the specimen with the most orthogonal data, average anterior/posterior kinematic error was calculated at only 2.1 ± 0.6 mm anterior; medial/lateral error at 1.6 ± 1.1 mm medial; superior/inferior error at 0.8 ± 0.4 mm inferior; internal/external error at 0.1 ± 9.6 ° external; flexion/extension error at 1.0 ± 4.2 ° flexed; and ad/abduction error at 3.0 ± 1.0 ° abducted compared to the input kinematics. Correlation coefficients with the corresponding load data were much lower, with a maximum coefficient of only 0.47 for anterior deviation compared with compressive load.
Figure 4.4. Comparisons of calculated and target anatomic positions of the tibial coordinate system with respect to the femoral coordinate system in each of the 3 specimens resulting in orthogonal bony coordinate systems for at least one time point during gait. Deviations from target kinematics were greatest during periods of increased flexion.
Figure 4.5. Average dynamic knee forces and torques of the 3 orthogonal specimens, normalized to body weight, throughout a simulated gait cycle with standard deviation error bars. Heel strike corresponds to 0% and 100% gait. Toe-off occurs at 64% of gait. Heel strike and push-off demonstrate the highest loading values during gait.

Discussion

Although verified calibration and calculation techniques were used to complete and analyze this study, only half of the specimens tested generated usable results. This was due to errors in orthogonality of mathematically reconstructed bony coordinate systems during gait simulation, leaving true anatomic position impossible to accurately calculate. These errors may have been generated in two ways: 1) While CMM data confirmed orthogonality during limb setup
at zero load, bone bending could cause landmark positions to shift relative to one another during testing, leading to physical orthogonality disparities; 2) The method of calculating and placing anatomical landmarks into the marker coordinate system was not accurate enough to maintain orthogonality.

Because orthogonality errors were not accompanied by an increase in force or torque at the joint, a mathematical error is more probable. With residuals of almost 1 mm in magnitude in the Vicon system, the inherent system error may have easily been high enough to promote inconsistencies and calculation error when solving for landmark positions. However, these landmark position calculations could not be avoided in the current study, as the working envelope of the CMM prevented the marker and robotic coordinate systems from being dually measured by the same equipment.

Even with computational challenges, a subset of data was successfully presented which compares actual kinematic output with target values during simulations of anatomic motion. Results showed that kinematic deviations were not significantly linked with the amount of load experienced at the knee joint. In fact, most deviation occurred during flexed periods of gait when loads were low – namely mid stance and swing. These errors may also be explained by the flexion of the specimen interfering with the line of sight between one or more markers and the more posteriorly positioned cameras.

Results suggest that the kinematics of simulated activities producing high loads at the joint are not at an increased risk to be influenced by bone bending and/or robotic compliance. Periods of high-loading did not correspond to decreased orthogonality, nor did they correspond to increases in kinematic deviation. However, future studies employing higher resolution equipment are necessary to confirm these findings.
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Chapter 5

Primary and secondary restraints of human and ovine knees for simulated in vivo gait kinematics

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Abstract

Purpose: Knee soft tissue structures are frequently injured, leading to the development of osteoarthritis even with treatment. Understanding how these structures contribute to knee function during activities of daily living (ADLs) is crucial in creating more effective treatments. This study was designed to determine the role of different knee structures during a simulated ADL in both
human knees and ovine stifle joints. **Methods:** A six degree-of-freedom robot was used to simulate each species’ in vivo gait kinematics while measuring three-dimensional joint forces and torques. Using a semi-randomized selective cutting method, we determined the primary and secondary structures contributing to the forces and torques along and about each anatomical axis. **Results:** In both species, the bony interaction, ACL, and medial meniscus provided most of the force contributions during stance, whereas the ovine MCL, human bone, and ACLs of both species were the key contributors during swing. This study contributes to our overarching goal of establishing functional tissue engineering parameters for knee structures by further validating biomechanical similarities between the ovine model and the human to provide a platform for measuring biomechanics during an in vivo ADL. These parameters will be used to develop more effective treatments for knee injuries to reduce or eliminate the incidence of osteoarthritis.

**Introduction**

Current treatments for the frequently injured soft tissue structures in the knee do not fully restore normal biomechanical properties, which contribute to long term joint degeneration. After surgical treatment and rehabilitation for severe knee injuries (e.g. ACL and meniscus tears), patients still experience the early onset of osteoarthritis 80,91. One factor contributing to joint degeneration is the surgeon’s inability to restore the native knee kinematics 92. This clinical problem stems from our limited understanding of normal knee biomechanics during actual activities of daily living (ADLs). Thus, surgeons must repair soft tissue structures with little knowledge of the function of these structures during ADLs.

Though investigators have provided important information about knee kinematics during ADLs 89,93, our current understanding of knee loading is usually limited to non-physiologic motions such as simulated clinical examinations (e.g. pivot shift test). Historically, the restraining
roles of knee structures have primarily been determined from laxity tests. Although investigators have also attempted to use strain gauges and force probes to estimate in vivo tissue forces, the inability to isolate structures for sensor calibration has prevented researchers from accurately measuring soft tissue forces in humans. Recently, Gill et al. estimated changes in human anterior cruciate ligament (ACL) forces by recording in vivo ACL elongation measurements during a single leg lunge activity at various flexion angles. To determine the in vivo ACL force, the elongation data was matched to force-elongation curves acquired from uni-axial tensile tests performed on cadaveric human limbs at various flexion angles. While this study increases our understanding of knee ligament forces for more complex activities, the investigators simulated a quasi-static activity and utilized non-physiologic tensile tests to estimate in vivo ligament forces. The field lacks knowledge of the forces and torques in the knee for ADLs.

While determining the required functional tissue engineering parameters (FTEPs) in a human is not practical, establishing and characterizing an animal model provides a first step in determining the forces and torques in the intact knee and for individual structures during ADLs. We have chosen to use the ovine model because prior work has shown that the ovine stifle is a valid surgical model for the human knee and is a suitable experimental model for studying various orthopaedic conditions and treatments for the knee.

The objective of this study was to determine the role of various knee structures during simulated gait kinematics for both the ovine stifle and human knee joints and to compare the role of each structure across species. The results of this study will help in determining the utility of the ovine model as a biomechanical surrogate for the human knee. Our long term research goal is to establish FTEPs for ADLs to serve as design criteria and evaluation benchmarks for traditional and novel treatment strategies.
Methods

Experimental Design

Three left and six right hind limbs (no pairs) from skeletally mature, mixed breed, female sheep (3-4 yrs; 50-78 kg) were included in the study along with four left and two right lower limbs (no pairs) from human cadavers (4 female, 2 male, 83±4 (SEM) yrs). However, two cadaver limbs transmitted no load through the ACL during testing, indicating abnormal physiology, and were excluded from the study. The remaining ovine (N=9) and human (N=4) limbs were testing using a six degree-of-freedom (DOF) robot (KR210; Kuka Robotics Corp., Clinton Township, MI) equipped with a six-axis load cell (Theta Model FT5498; ATI Industrial Automation, Apex, NC). The robot simulated 6 DOF species specific gait motions derived from ovine \textsuperscript{105} and human subjects \textsuperscript{89}, while recording corresponding joint forces and torques. Once a specimen was fixed to the robot end effector, the knee joint was cycled through its species specific gait during a selective cutting protocol, as described in detail below. Resulting changes in force and torque allowed us to rank the loading contributions of each structure during simulated gait.

Sample Preparation and Setup in Robot

All limbs were stored at -20°C until the evening before testing. Each knee was dissected free of all muscles and tendons, leaving the joint capsule, the collateral and cruciate ligaments, and the two menisci intact. The tibia was rigidly attached and aligned with the load cell and robot end effector axes according to the tibial joint coordinate system \textsuperscript{84}. The tibial joint center point was digitized using a coordinate measurement machine (CMM, Faro Digitizer F04L2, FARO Technologies Inc., Lake Mary, FL), and all rotations, translations, forces, and torques were applied and recorded about this point. Landmarks on the collateral ligaments and along the mechanical
axes of the tibia and femur were used to define and measure the position of the knee during setup and testing.

Due to differences in kinematics and information available in the Tapper (ovine) and Lafortune (human) manuscripts, the remaining test setup procedures to achieve the gait starting position were species dependent.

In ovine testing the knee was placed at a 60.5° flexion angle, corresponding to the midpoint of joint flexion during swing phase, as reported by Tapper et al.\textsuperscript{105}. This starting flexion angle was selected as we postulated that the forces and torques are minimal as the knee moves toward peak flexion. Small translational changes were made to minimize the forces and torques to <5 N and <1 Nm, respectively, to achieve an unloaded state. The ovine gait kinematics were cycled from this starting position in swing.

In human testing, the knee was placed at the position of peak flexion during stance, as reported by Lafortune et al.\textsuperscript{89}. The orientation of the knee was adjusted until all three rotations were within ±0.5° of the Lafortune motion. At this position, a 500N compressive force was applied until the load could be maintained following several cycles of simulated gait to simulate compression experienced at peak flexion during stance based on unpublished data acquired from the Hewett biodynamics group, focused on knee injury prevention.

A more detailed description of how each limb was prepared, secured into test fixtures, and placed into its starting position can be found in previous reports\textsuperscript{69,106}.

\textit{Simulated 6 DOF In Vivo Motion Robot Testing}

All tests were performed at room temperature with the joint wrapped in saline soaked gauze to prevent soft tissue dehydration. Ovine motions were applied to ovine specimens and human
motions were applied to human specimens. To minimize viscoelastic effects, an initial set of 10 gait cycles was applied to each knee, followed by another 10 cycles to record the forces and torques of the intact joint. A structure was then selectively cut at random (e.g. MCL), and 10 cycles of simulated gait kinematics were repeated to record the new joint forces and torques. This process was repeated in random order to reduce the effect of tissue interactions until each of the posterior cruciate ligament (PCL), medial collateral ligament (MCL), lateral collateral ligament (LCL), lateral meniscus (LM), medial meniscus (MM), medial capsule (MedCap), and lateral capsule had been cut. Each test always concluded with the elimination of the bony interaction followed by the removal of the ACL. The ACL-isolated condition allowed us to measure forces and torques with only the ACL transmitting load across the joint. Finally, the ACL was removed, and the motions were applied with the tibia rotating freely in space. The resulting forces and torques due to gravity and robot inertia were then subtracted from each previously run test. The reduction in force and torque from the removal of a given structure was used to determine the primary and secondary structures contributing to loads in each anatomical DOF.

Data Analysis

Forces and torques were recorded in the tibial reference frame based on the knee joint coordinate system established by Grood and Suntay (1983). Forces correspond to the anterior-posterior, medial-lateral, and compression-distraction anatomical axes. Torques correspond to the adduction-abduction, flexion-extension, and internal-external moment convention. The 8th and 9th cycles of each 10 cycle test were used for analysis to reduce cycle effects. Data were averaged across specimen over a normalized gait cycle (%) for the intact condition and each selectively cut condition. The load contribution of each structure was determined by computing the changes in
force and torque along and about each anatomical axis due to the removal of that structure. The average change in force or torque was then compared against the average intact condition to calculate the percent contribution in each anatomical DOF. The structures were then ranked according to the most significant contributors during both stance phase (heel strike, mid stance, and push-off) and swing phase (peak flexion). A primary or secondary contributor to load in our position controlled test translates to a primary or secondary restraint to in vivo motion.

Statistical Analysis

For each point of interest, a two-tailed Student’s t-test with a test value equal to zero was used to examine the contribution (\% of intact load) of the individual structures between species. In all cases, to be considered a structural contributor, the force or torque value recorded after cutting the structure must have shown a statistically significant difference from the intact condition while also representing a physiologically significant change. Therefore, thresholds for physiologic significance were set at 10\% of the intact force or torque and \( >5N \) of force or \( >1Nm \) of torque. Overall rankings for stance phase contribution were assigned for each structure by considering loads with the highest percent of intact contribution among the three stance phase points. Rankings for swing phase contribution were assigned by only considering loads at peak flexion. Structures were designated as secondary contributors if their percentage of intact load was more than 5\% lower than the primary contributor(s). All data were normal. Significance level for all comparisons was set at \( p < 0.05 \).
Results

Stance Phase

The primary structural contributors during the stance phase of gait were similar between the human and ovine knees (Table 5.1.: Primary). Both species demonstrated compression as the primary direction of load. The total force recorded in the ovine stifle during stance was approximately 300N, while in the human knee approximately 500N were recorded (Figures 5.1. and 5.2.: Stance). Of note, the ACL was the primary restraint to anterior tibial translation and bony interaction provided primary restraint to posterior, medial and compressive translations as well as adduction and internal rotations.

There were also similarities among the secondary contributors during the stance phase between the two species (Table 5.1.: Secondary). Particularly, the ACL resisted medial translation while the medial meniscus was a secondary contributor to posterior and compressive translations. Interestingly, in the ovine knee, the only secondary structural contributors were the ACL and medial meniscus, while the human knee had many secondary contributors (Figure 5.1.: Stance). In addition, there were no significant contributions from the PCL, LCL, or lateral capsule, nor did any structure function to resist distraction or external rotation during stance in either species.
Table 5.1. Force and torque contributions of knee structures during the stance phase of gait.

Ovine and human ACLs, MMs, and bony interactions have similar restraining roles, particularly in the translational degrees of freedom.
Figure 5.1. Human total force during gait. Sum of the average translational components of intact knee force and the corresponding drop in all structurally deficient knee forces during the gait cycle in the human knee joint. Human toe-off occurs at 64% of gait, though loading continues to be compressive. Bony interaction accounts for the majority of total load in stance and swing, followed by the medial meniscus.
Swing Phase

During the swing phase of gait, there were fewer similarities among the primary restraints between the human and ovine knees (Table 5.2.: Primary). The primary loading direction in the ovine stifle was distraction, while the human knee remained overall compressed. The total force recorded in the ovine stifle during swing was approximately 100N, while in the human knee approximately 200N were recorded (Figures 5.1. and 5.2.: Swing). In both species the ACL resisted anterior and medial tibial translation. However, in the human knee, only the bony interaction provided the remaining primary contributions, while in the ovine knee the menisci, bony interaction, and the MCL were all primary contributors.

There were no similarities between the two species related to secondary contributors (Table 5.2.: Secondary). In addition, there were no significant contributions made by the LCL, medial, or lateral capsule, nor did any structure function to resist internal rotation during swing in either species.
Table 5.2. Force and torque contributions of knee structures during the swing phase of gait. The ACL continues to act as a restraint to anterior translation and medial translation in both species throughout gait. The ovine MCL becomes a significant restraint in multiple degrees of freedom while the human MCL remains functionally absent.

<table>
<thead>
<tr>
<th>Contributors in Swing</th>
<th>Sheep</th>
<th></th>
<th>Human</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Primary</td>
<td>Secondary</td>
<td>Primary</td>
</tr>
<tr>
<td>Translations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anterior</td>
<td>ACL*</td>
<td>MCL***</td>
<td>ACL*</td>
</tr>
<tr>
<td>Posterior</td>
<td>Bone, LM</td>
<td>LM</td>
<td>Bone</td>
</tr>
<tr>
<td>Medial</td>
<td>ACL</td>
<td>MCL</td>
<td>Bone</td>
</tr>
<tr>
<td>Lateral</td>
<td>MCL</td>
<td>Bone</td>
<td>Bone</td>
</tr>
<tr>
<td>Compression</td>
<td>LM</td>
<td>ACL*, PCL</td>
<td>Bone</td>
</tr>
<tr>
<td>Distraction</td>
<td>MCL</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adduction</td>
<td>ACL*</td>
<td>MCL***</td>
<td>Bone</td>
</tr>
<tr>
<td>Abduction</td>
<td>MCL***</td>
<td>Bone</td>
<td>Bone</td>
</tr>
<tr>
<td>Flexion</td>
<td>MCL***, ACL</td>
<td>Bone</td>
<td>Bone</td>
</tr>
<tr>
<td>Extension</td>
<td>Bone</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal</td>
<td>MCL***</td>
<td></td>
<td></td>
</tr>
<tr>
<td>External</td>
<td>MCL***</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* denotes statistical difference between species

* $p < 0.05$ ; ** $p < 0.01$ ; *** $p < 0.001$ in comparing % intact loads
Figure 5.2. Ovine total force during gait. Sum of the average translational components of intact knee force and the corresponding drop in all structurally deficient knee forces during the gait cycle in the ovine stifle joint. Ovine toe-off occurs at 68% of gait when loading is primarily distractive. Bony interaction accounts for the majority of total force in stance, followed by the medial meniscus. The MCL and ACL account for the majority in swing.
Discussion

Analysis of the stance phase of gait reveals more similarities between species than in the swing phase of gait. The combination of the bony interaction, menisci, and ACL accounted for almost all of the primary and secondary stabilization in the knee during stance in both species. As expected, the ACL was a major restraint to anterior and medial tibial translations in both species, which is supported by previous studies \(^1,46,54,107\). Similarly, the medial meniscus was a primary restraint to lateral translation in both species. This could have been caused by the superficial meniscal ridge contacting the medial condyle as the tibial moved laterally. The human medial meniscus also functioned to resist adduction, as the ovine medial meniscus did, and as has been previously reported \(^108-110\). The largest difference between the species during stance was the role of the lateral meniscus as a restraint to translation. Both the medial and lateral human menisci resisted posterior and lateral translations, while the sheep lateral meniscus did not resist any translation. This could possibly be due to differences in bone morphology.

The large load contributions from the bony interactions during stance phase demonstrate how the compressed state of the knee can shield soft tissue structures from loading. To our knowledge, only one previous biomechanical study investigated the bony interaction’s contribution to forces and torques in the knee. In a study by Sakane et al. \(^54\), comparisons of anterior loads generated during anterior tibial translation showed that the bony interaction was a secondary restraint to anterior translation at flexion angles beyond 30°. This disputes our results that found bone resists posterior translations. However, this study applied pure anterior displacements in passive flexion positions at zero-load, which does not represent the native mechanical environment of the knee, as the hamstrings pull the tibia further posteriorly during flexion activities. In addition, this previous study, along with the majority of previous knee biomechanical studies, does not apply physiologic joint loads, minimizing compression and the
role of bony interaction. Still, both studies suggest that load sharing in the knee joint occurs which can impact repair mechanics and design criteria.

During the swing phase of gait, the ACL remained the primary restraint to anterior tibial translation in both species, further demonstrating the ACL dependence of both human and ovine knees. As expected, in ovine knees, the bony interaction contributed less compared to stance phase, while human bony interactions played similar roles to stance phase. This was due to the compressive knee state measured for the human knee. Another key difference between the species during swing was that the ovine MCL became far more active than the human MCL while the ovine medial meniscus was less critical. This phenomenon could be explained by the sheep’s “knock-kneed” gait which naturally exposes the ovine knee to higher levels of abduction. Differences in knee laxity between species also became apparent during swing, as no structure of the human knee functioned to resist lateral translation, abduction, or external rotation. In each of these DOFs, the ovine MCL played the primary role.

One limitation of this study was the small sample size (N=4) for human cadaveric testing. Though the sample size would ideally be larger for cadaveric studies, results were consistent across specimen and the total load coefficient of variation was less than 22% during stance phase. Furthermore, the loads of the intact and ACL-only conditions (average 41.1N ± 20.6N) align closely with unpublished data from a separate study comparing intact and ACL-deficient conditions (N=4, average 42.9N ± 17.3N), also generated by our lab. Comparisons of these two unique groups of specimens showed that the total force in the ACLs of this study was not statistically different from the total force contribution of the unpublished ACL data, suggesting that the human specimens used in this study are representative of the population, not outliers, and that the contributions determined are representative of the population.
Other limitations of this study included the necessity to apply averaged kinematics from one study to a different set of knee specimens, and that among those knee specimens there is inherent population variability. We acknowledge that the application of an averaged motion to joints of different sizes could produce variability in the sample population. To examine the effect of this variability on study outcomes, the analysis of structural contribution to force and torque was repeated by internally ranking each specimen before combining the results, which did not affect our conclusions. Furthermore, there was minimal variability in measured loads between subjects (Figures 1 and 2). Therefore, it is unlikely that this limitation would invalidate resulting rank order of each restraint, though it may help explain the differences seen between ovine and human structures in contributing to percent total intact force. In future studies, we plan to use the ovine model to investigate this limitation further, by applying both subject-specific and averaged motions to each specimen to determine how averaged motion paths may affect the measured kinetics. One solution may be to utilize the robot’s load control capabilities to apply individualized kinematics based on specimen specific parameters and load boundaries. This knowledge would be useful to apply to human cadaveric testing, as we are currently unable to collect reliable in vivo load measurements and must always rely on in vitro testing using one averaged or representative motion for an entire cohort of specimens.

Results of this study support use of the ovine stifle joint as a biomechanical model for human knee dynamics, along with other previously reported studies\(^8,11\), as the loaded structures in the knee during stance are similar between species. Concurrent studies in our lab may further validate the ovine model by investigating knee ligament strains and kinematic response to injury. By using the ovine model as a surrogate for measuring in vivo and in vitro biomechanical function, researchers may be able to further develop the functional standards needed for designing repair
procedures. This model may also serve to evaluate the safety and efficacy of future repair strategies.

Normal knee function during gait depends on the restraining roles of the soft tissue structures and bony interaction during normal activities. While previous studies provide valuable information about the roles of knee structures, they have not presented critical information about their functions during an ADL. Knowledge of the contributions of each structure during ADLs will allow investigators to better understand the normal biomechanics of the knee joint. For example, as it applies to ACL research, ovine and human specimens both demonstrated that in addition to its primary role as a restraint to anterior movement the ACL also acts as a restraint to medial translation. This additional role may be key a consideration when designing materials and techniques for ACL repair. By exposing tissues to the mechanical context encountered during normal activities, researchers can better establish the patterns and limits of expected usage which govern functional tissue engineering parameters (FTEPs). These FTEPs will provide crucial design criteria for the development of more effective strategies for the prevention and treatment of knee injuries.

Acknowledgements

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Chapter 6

Effects of population variability on knee loading during simulated human gait

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Abstract

Purpose: With the invasive nature of in vivo sensing in patients, cadaveric models offer researchers an alternative way of studying native tissues in situ for simulated activities and scenarios. However, as these tests are conducted using donor specimens with unmatched kinematics, techniques imposing a population average of human motion will induce some degree of deviation from true physiologic conditions. The specific influences deviations from true in vivo kinematics have yet to be fully characterized, and recorded kinetic data continues to vary widely between specimens. Therefore, this study was designed to identify factors which explain the kinetic variability observed during robotic simulations of a single human gait motion using a
sample of human cadaver knees. **Methods:** Twelve (12) fresh frozen human cadaver limbs (4 male, 8 female, 58 ± 16 yrs) were subjected to geometrical analysis of the tibio-femoral joint and cyclical stiffness testing during which specimens were cycled between ± 100 N and ± 10 N-m in each anatomical degree of freedom (DOF). A simulated gait motion was then applied to each specimen to record the 6-DOF forces and torques. Resulting kinetics, specimen geometries, and various representations of the stiffness data were first reduced to functional attributes using principal component analysis and fit to a generalized linear prediction model. This model revealed properties of the knee that lead to the most variation in joint kinetics for a simulated gait motion.

**Results:** The capacity of knee articulation topography to generate compressive force in the joint was the largest contributor to kinetic variation, which included increases in compression during stance phase. Overall joint size, femoral notch height, translational laxity, and ad/abduction stiffness also significantly contributed to kinetic variation, which additionally included medial/lateral and anterior/posterior forces and associated torques. Future studies will investigate customizing kinematic paths based on these properties to better simulate native conditions and reduce sampling variation, improving biomechanical test methods and evaluation strategies for future orthopaedic techniques.

**Introduction**

Current methods of repairing or reconstructing structures of the knee continue to result in long-term failure and/or premature onset of osteoarthritis. This is likely caused by the inability of surgeons to reproduce in vivo functionality, as these functional requirements have not yet been adequately quantified for in vivo physiologic scenarios. Measuring function in vivo is invasive and there is the risk that the sensing equipment itself alters the native environment. Therefore, to
establish benchmarks and design criteria and to evaluate the functionality of repairs, researchers have turned to in vitro methods to assess the functionality of native knee structures in situ\textsuperscript{32,67,68}.

By applying kinematic simulations of activities of daily living (ADLs) to tissues in vitro, researchers can begin to develop functional tissue engineering parameters (FTEPs) for knee structures in a physiologic context. This requires that test methods reproduce physiologic conditions as closely as possible to represent native functionality. However, in the testing of human tissues, cadaver specimens are pooled from a wide variety of donors and encompass a vast population, even when specific population requests are made (e.g. age or gender). Therefore, a generalized test method using only averaged kinematic data from the population will likely not achieve accurate physiological representation for every donor, increasing variability of measured tissue properties.

Wide variability among the donor population can lead to statistically insignificant results in studies aimed at defining native functionality, and can mask treatment effects in studies evaluating repairs\textsuperscript{112}. Multiple research groups have reported difficulty in managing variability between and within treatment groups when measuring such physiologic attributes as joint kinematics and kinetics\textsuperscript{113}. These difficulties are even present in highly controlled animal studies\textsuperscript{114}.

To mitigate the effects of population variability, researchers must first identify which donor attributes lead to wide variations in the physiologic properties of interest to tailor in vitro tests towards meeting native circumstances and constraints for each specimen. Physical properties (such as anatomic geometry) and material properties (such as tissue stiffness) may be impacting studies so that the test methods do not represent a physiologic condition for each specimen. Therefore, this study aimed to identify which properties had the greatest contributions to sample
variability in kinetics for a standard simulated gait motion. Effects of human tibio-femoral geometry and 6-degree-of-freedom (6 DOF) knee stiffness on 6 DOF kinetic outputs were considered.

Results showed that strong correlations exist between knee geometry and stiffness and resulting loads for an intact knee. Top predictors included overall size, femoral notch height, overall joint laxity, and ad/abduction rotational stiffness. Results are supported by similar findings in the field of kinematic measurement. This study provides valuable clinical insight into how population variability may affect functional demands of patients and the ultimate outcomes of potential repairs.

**Methods**

*Anatomic Geometry*

Twelve (12) fresh frozen human cadaver limbs (4 male, 8 female, 58 ± 16 yrs, 171 ± 49 lbs) were used in this study. Specimens were kept frozen at -20°C until the night before testing. Thawed specimens were dissected free from all soft tissues, leaving the cruciate and collateral ligaments, menisci, and joint capsule intact. Using a coordinate measurement machine (CMM, Faro Digitizer F04L2, FARO Technologies Inc., Lake Mary, FL), anatomic landmarks were probed to measure pre-determined geometries of interest and to define the joint coordinate system according to Grood and Suntay. Landmarks were selected on both the tibial plateau and femoral condyles to encompass anatomic descriptors of the entire tibio-femoral interaction (Tables 6.1. and 6.2.: Landmarks). From these landmarks, a custom MATLAB program calculated dimensions and surface areas suspected to most influence gait kinetics (Tables 6.1. and 6.2.: Geometries).
<table>
<thead>
<tr>
<th>Tibial Landmark</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak of the Medial Spine (Tibial Center Point)</td>
<td>MSP</td>
</tr>
<tr>
<td>Peak of the Lateral Spine</td>
<td>LSP</td>
</tr>
<tr>
<td>Anterior Ridge of the Medial Compartment</td>
<td>MCAR</td>
</tr>
<tr>
<td>Anterior Ridge of the Lateral Compartment</td>
<td>LCAR</td>
</tr>
<tr>
<td>Posterior Ridge of the Medial Compartment</td>
<td>MCPR</td>
</tr>
<tr>
<td>Posterior Ridge of the Lateral Compartment</td>
<td>LCPR</td>
</tr>
<tr>
<td>Medial Ridge of the Plateau</td>
<td>MR</td>
</tr>
<tr>
<td>Lateral Ridge of the Plateau</td>
<td>LR</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Calculated Geometries</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area of the Medial Compartment</td>
<td>MSP, MCAR, MCPR, and MR as boundaries</td>
</tr>
<tr>
<td>Area of the Lateral Compartment</td>
<td>LSP, LCAR, LCPR, and LR as boundaries</td>
</tr>
<tr>
<td>Total Area of the Plateau</td>
<td>MCAR, LCAR, MCPR, LCPR, MR, and LR as boundaries</td>
</tr>
<tr>
<td>Width of the Medial Compartment</td>
<td>MSP to MR</td>
</tr>
<tr>
<td>Width of the Lateral Compartment</td>
<td>LSP to LR</td>
</tr>
<tr>
<td>Total Width of the Plateau</td>
<td>MR to LR</td>
</tr>
<tr>
<td>A/P Depth of the Medial Compartment</td>
<td>MCAR to MCPR</td>
</tr>
<tr>
<td>A/P Depth of the Lateral Compartment</td>
<td>LCAR to LCPR</td>
</tr>
<tr>
<td>Average A/P Depth of the Plateau</td>
<td>$\frac{(\text{MCAR to MCPR} + \text{LCAR to LCPR})}{2}$</td>
</tr>
</tbody>
</table>

**Table 6.1.** Tibial landmarks and geometries.
<table>
<thead>
<tr>
<th><strong>Femoral Landmark</strong></th>
<th><strong>Abbreviation</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Femoral Notch</td>
<td>FN</td>
</tr>
<tr>
<td>Peak of the Medial Interior Ridge</td>
<td>MIRP</td>
</tr>
<tr>
<td>Peak of the Lateral Interior Ridge</td>
<td>LIRP</td>
</tr>
<tr>
<td>Distal Medial Condyle</td>
<td>DMC</td>
</tr>
<tr>
<td>Distal Lateral Condyle</td>
<td>DLC</td>
</tr>
<tr>
<td>Medial Condyle Arc</td>
<td>MCA</td>
</tr>
<tr>
<td>Lateral Condyle Arc</td>
<td>LCA</td>
</tr>
<tr>
<td>Anterior Ridge of the Medial Condyle</td>
<td>ARMC</td>
</tr>
<tr>
<td>Anterior Ridge of the Lateral Condyle</td>
<td>ARLC</td>
</tr>
<tr>
<td>Posterior Medial Condyle</td>
<td>PMC</td>
</tr>
<tr>
<td>Posterior Lateral Condyle</td>
<td>PLC</td>
</tr>
<tr>
<td>MCL Origin</td>
<td>MCLO</td>
</tr>
<tr>
<td>LCL Origin</td>
<td>LCLO</td>
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<thead>
<tr>
<th><strong>Femoral Geometries</strong></th>
<th><strong>Definition</strong></th>
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<tr>
<td>Height of the Medial Condyle</td>
<td>FN to DMC</td>
</tr>
<tr>
<td>Height of the Lateral Condyle</td>
<td>FN to DLC</td>
</tr>
<tr>
<td>Average Notch Height</td>
<td>(FN to DMC + FN to DLC) / 2</td>
</tr>
<tr>
<td>Notch Width</td>
<td>MIRP to LIRP</td>
</tr>
<tr>
<td>Medial Condyle Radius of Curvature</td>
<td>Calculated from Arc</td>
</tr>
<tr>
<td>Lateral Condyle Radius of Curvature</td>
<td>Calculated from Arc</td>
</tr>
<tr>
<td>Depth of the Medial Condyle</td>
<td>ARMC to PMC</td>
</tr>
<tr>
<td>Depth of the Lateral Condyle</td>
<td>ARLC to PLC</td>
</tr>
<tr>
<td>Average Depth of the Condyles</td>
<td>(ARMC to PMC + ARLC to PLC) / 2</td>
</tr>
<tr>
<td>Width of the Medial Condyle</td>
<td>MIRP to MCLO</td>
</tr>
<tr>
<td>Width of the Lateral Condyle</td>
<td>LIRP to LCLO</td>
</tr>
<tr>
<td>Total Width of the Condyles</td>
<td>MCLO to LCLO</td>
</tr>
</tbody>
</table>

Table 6.2. Femoral landmarks and geometries.
Recording 6 DOF Stiffness

Specimens were then placed on the end effector of a 6 DOF robot (KR210; Kuka Robotics Corp., Clinton Township, MI) for testing. Using the CMM, the coordinate system of the tibia was aligned to a 6-axis load cell (Theta Model FT5498; ATI Industrial Automation, Apex, NC) attached the end effector and the femur was rigidly fixed to a testing platform, allowing the robot to manipulate the tibia about the femur. The limb was then placed at 60 degrees of flexion, corresponding to the peak flexion during the swing phase of gait. This flexion angle was chosen to minimize the risk of meniscal impingement and maximize the resolution of forces and torques measured, as laxity increases with flexion. Once at 60 degrees, a custom written force/torque controlled program explored the passive envelope of the knee between ± 100N and ± 10 Nm until the center of laxity in each of the remaining DOFs was achieved. The program then cycled the knee between positive and negative limits in each DOF, recording simultaneous loadings and displacements (Figure 6.1.). This resulted in 6 sets of 6 hysteresis curves, one set for each of the 6 directions of motion during which loads in all 6 DOFs were recorded, including those that did not experience any displacement. Stiffness terms describing load changes in the same DOF of motion are referred to as main stiffnesses, and stiffness terms describing load changes in a DOF other than the direction of motion are referred to as cross-coupled stiffnesses.
Figure 6.1. Cyclic stiffness testing. The tibia cycles between ± 100N and ± 10 Nm in each DOF on at a time while corresponding 6 DOF loads are recorded. In this test, the blue line represents the test direction. Note that the highest loads may not be observed in the direction of motion.

Recording Gait Kinetics

All specimens underwent identical gait kinematics as recorded by Lafortune et. al (Figure 6.2.). Following stiffness testing, specimens were positioned to the orientation corresponding to peak flexion during mid-stance. Translations were then adjusted to produce a zero force condition before applying one cycle of gait motion. Compressive load was then added until peak compression during gait reach two times body weight, the average load experienced by patients who underwent a total knee replacement. Further details of the setup procedure can be found in previous reports.
Figure 6.2. Reproduction of gait kinematics as recorded by Lafortune et al. Translations correspond to anterior/posterior (A/P), medial/lateral (M/L), and compression/distraction (C/D). Rotations correspond to internal/external (I/E), flexion/extension (F/E), and adduction/abduction (Ad/Ab). Heel strike occurs at 0 and 100% gait. Toe-off occurs at 64% gait.

Each specimen was cycled through gait 20 times, with only the last 10 cycles being analyzed to minimize viscoelastic effects. During cycling, the load cell captured the forces and torques experienced by the intact knees about the joint center point, which was defined as the peak of the medial tibial spine.

Geometrical Analysis

Calculated dimensions of both the tibia and femur were combined to compose a data set of 21 geometries for each specimen. To reduce degrees of freedom and to identify the driving source
of geometric variation, principle component analysis (PCA) was performed using the “prcomp”
function within the built-in stats package of R 3.0.2. The first two principle components,
accounting for 80% of the geometric specimen variability, were chosen to be representative of
geometric differences in further analyses (see Predictive Modeling).

**Stiffness Analysis**

Stiffness hysteresis curves were recorded as load (N or Nm) over distance (mm or degrees
of rotation) (Figure 6.3.). Due to highly non-linear response during cyclic stiffness testing, 5
different methods were used to analyze the resulting hysteresis curves.

![Stiffness Hysteresis Curves](image)

**Figure 6.3.** Representative set of hysteresis curves following stiffness testing. Zero
displacement represents the center of laxity at 60 degrees of flexion. 6 DOF forces resulting
from pure motion in each of the 6 DOFs make up a set of 36 curves. Note the nonlinearities
present, particularly in compression/distraction forces, and large cross-coupled loading in the stationary DOFs.

1. Symmetric Linearized

Edwards et. al has previously described a method used to discretely quantify stiffness of spinal segments\textsuperscript{116}. This method utilizes matrix algebra and the method of least squares to identify linear stiffness coefficients from hysteresis data. It results in a calculated 6x6 stiffness matrix representing the relationship between load and displacement in each anatomical DOF. However, the solution requires that the resulting stiffness matrix be symmetric. Therefore, data points from each cross-coupled curve were combined with inverse cross-coupled values (e.g. A/P force during M/L motion with M/L force during A/P motion) to calculate the linear stiffness estimates describing the overall loading interactions of 2 DOFs. Using this method, a total of 21 terms were generated, which could be arranged into a symmetric stiffness matrix, describing the relationship of force and displacement for each specimen. These terms were then subjected to PCA, as described above. The first three principle components, accounting for 82\% of the variability in stiffness, were chosen to be representative of specimen stiffness differences in further analyses (see Predictive Modeling).

2. Linearized

This method utilized the “polyfit” function of MATLAB R2012a to estimate a linear fit to each of the hysteresis curves. This allowed cross-coupled terms to remain separate from their inverses so that linearized stiffness values were estimated for each main and cross-coupled hysteresis curve. This resulted in a total of 36 terms generated which were also subjected to PCA.
The first three principle components, accounting for 74% of the variability, were chosen to be representative of stiffness differences in further analyses (see Predictive Modeling).

3. Split Linearized

This method is the same as above except that both main and cross-coupled terms were split into their positive and negative components of cyclic motion (e.g. anterior movement was considered separately from posterior movement). This broke the hysteresis curves apart at the center of laxity, and estimates of linearized stiffness coefficients were calculated for each segment using the polyfit function. A total of 72 terms were generated and subjected to PCA. The first three principle components, accounting for 63% of the variability, were chosen to be representative of stiffness differences in further analyses (see Predictive Modeling).

4. Averaged Split Linearized

After split linearized terms were generated, both positive and negative halves of the hysteresis curves were brought together again by averaging the absolute values of each side. While this technique allowed stiffness data to be represented in fewer terms, it resulted in missing sign data. A total of 36 terms were generated and subjected to PCA. The first three principle components, accounting for 68% of the variability, were chosen to be representative of stiffness differences in further analyses (see Predictive Modeling).

5. Spline Modeling

To better represent the knee’s frequently nonlinear response to uniaxial motion, each of the 36 hysteresis curves generated during stiffness testing were modeled with sequential 4th order beta
splines using the functional data analysis, “fda”, package of R 3.0.2.\textsuperscript{115,117} Hysteresis load data were converted to percent body weight (% BW) to normalize the models. Three nodes defined the models at the negative extremes, center of laxity, and positive extremes, allowing a total of 5 beta coefficients to fully describe and differentiate each of the hysteresis curves (Eq. 1, Figure 6.4.). High beta coefficients translate to high load values surrounding its location on the displacement axis. Betas 1 and 2 describe loads during the negative direction of motion while betas 4 and 5 describe loads during the positive direction of motion. The third beta coefficient was discarded, as it corresponded to the load at the center of laxity, which was normalized to zero for each of the models. Betas 1, 2, 4, and 5 from each of the 36 hysteresis models comprised a data set of 144 terms describing non-linear stiffness behavior. However, because stiffness testing cycled between constant load values which were then normalized to % BW, variation in all of the main stiffness hysteresis curves was driven by body weight and not factors of interest. For this reason, beta coefficients from main curves were excluded from this analysis. The remaining 120 terms were then subjected to the same methods of PCA. The first three principle components, accounting for 68% of the variability, were chosen to be representative of non-linear stiffness differences in further analyses (see Predictive Modeling).

\begin{equation}
Y = \beta_1 \ast b_1(X) + \beta_2 \ast b_2(X) + \beta_3 \ast b_3(X) + \beta_4 \ast b_4(X) + \beta_5 \ast b_5(X) + \varepsilon
\end{equation}

\text{Eq. 1}

Y is load (% BW)

X is displacement (% of total range)

b’s are constants defining the spline model of order 4 with 1 central node at 50% displacement

\varepsilon is the error (ignore)

β’s are the variables specific to each hysteresis test

Averaging β’s across specimen results in average stiffness curve
A. Raw Hysteresis Data

![Graph showing hysteresis curves for Ant/Post, Med/Lat, Comp/Dist, Int/Ext, Flex/Ext, and Ad/Abuct movements.]

B. Spline Model

![Graph showing %BW vs. % Change from Lateral to Medial Force with labeled Beta values 1 to 5.]

**Figure 6.4.** A. Recorded data and B. resulting spline models of a representative set of 6 hysteresis curves resulting from medial/lateral translation. Curves were normalized for the starting center of laxity to be located at 50% of the displacement and to be relative to any small...
residual forces recorded at the center of laxity due to viscoelastic effects. Beta coefficients of the model correspond to values at each of the 5 marked locations.

**Gait Simulation Analysis**

Gait dynamics were averaged over each cycle and expressed as load (% BW) across a normalized gait cycle (% Gait) for each of the 6 DOFs (Figure 6.5.). Metrics chosen for analysis were 1. Overall range of load, 2. Average load, 3. Load at heel strike, 4. Load at driving phase of stance, 5. Load at toe-off, and 6. Load at peak flexion. These metrics were stored for each of the 6 DOFs generating 36 total terms describing specimen gait dynamics. As with the previous specimen factors, these response measures were also subjected to PCA to reduce degrees of freedom and to identify the driving source of dynamic loading variation. The first three principle components, accounting for 80% of the variability, were chosen to be representative of dynamic gait loading differences in further analyses (see Predictive Modeling).
Figure 6.5. Resulting dynamics of each of the 12 tested cadaver knees.

Predictive Modeling

To identify potential significant contributors to dynamic variation in gait, several generalized linear models were explored using the “lm” function of the built-in stats package of R 3.0.2\(^\text{115}\). The first set used only the first 2 principle components (PCs) of geometric analysis to model each of the first 3 PCs of gait dynamics. The next set used only the first 3 PCs of each of
the stiffness analyses (one at a time) to model the 3 gait dynamics PCs. The next combined all geometric and stiffness PCs to model gait dynamics.

Finally, models were optimized to include only a subset of both the geometric and stiffness factor PCs by using the “leaps” package in R 3.0.2. This function performs an exhaustive search for the best subsets of a given set of potential regressors using a branch-and-bound algorithm. This allowed us to explore usage of each of the stiffness analysis techniques to identify the most appropriate for predicting dynamic loading response during physiologic motion.

Each model was evaluated by its adjusted $R^2$ term, calculating from it the Pearson’s correlation coefficient ($R$), and p-value. An alpha value of 0.05 or less was used to identify significant factors.

**Results**

*Principle Components*

PCA of gait dynamics showed that the first principle component (PC1) can be considered as the compression/distraction values during stance along with their associated anterior/posterior and ad/abduction loads. PC1 of the gait dynamics (G1) accounted for 31% of the total variation between specimens. PC2 of gait dynamics (G2), medial/lateral forces with associated torques, accounts for 25% of the total variation. Meanwhile, PC3 of gait dynamics (G3) can be considered the average anterior/posterior force with associated torques and accounts for 23% of the variation.

PCA of geometric properties of the tibio-femoral interaction revealed that the largest contributors to geometric variation were properties describing the overall size of the joint along with femoral notch height. PC1 of geometry (g1) can be considered a scale factor, accounting for 64% of the total variation and containing properties such as total area of the tibia plateau, condyle
depth, and condyle width. PC2 of geometry (g2) is clearly identifiable as a notch height factor, accounting for 16% of the variation and containing only lateral, medial, and average notch heights.

Results of the 4 linear stiffness estimation techniques showed consistent PCA results across each test. Each identified main and cross-coupled translational stiffness as the primary principle component of stiffness (s1), cross-coupled stiffness (especially between transverse and sagittal plane DOFs) as the secondary principle component (s2), and cross-coupled terms of adduction/abduction stiffness as the tertiary principle component (s3) (Table 6.3).
## PCA Summary of Linear Estimation Techniques

<table>
<thead>
<tr>
<th>1. Symmetric Linearized</th>
<th>Proportion of Variance</th>
<th>Top 3 Contributors*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC1</td>
<td>53%</td>
<td>A/P main</td>
</tr>
<tr>
<td></td>
<td></td>
<td>I/E and F/E cross-coupled</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A/P and C/D cross-coupled</td>
</tr>
<tr>
<td>PC2</td>
<td>16%</td>
<td>M/L and C/D cross-coupled</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M/L and F/E cross-coupled</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A/P and M/L cross-coupled</td>
</tr>
<tr>
<td>PC3</td>
<td>14%</td>
<td>A/P and Ad/Ab cross-coupled</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F/E and Ad/Ab cross-coupled</td>
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</table>

<table>
<thead>
<tr>
<th>2. Linearized</th>
<th>Proportion of Variance</th>
<th>Top 3 Contributors*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC1</td>
<td>35%</td>
<td>A/P main</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ad/Ab stiffness during M/L translation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C/D main</td>
</tr>
<tr>
<td>PC2</td>
<td>26%</td>
<td>F/E main</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C/D stiffness during M/L translation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C/D stiffness during F/E rotation</td>
</tr>
<tr>
<td>PC3</td>
<td>12%</td>
<td>Ad/Ab stiffness during C/D translation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ad/Ab stiffness during F/E rotation</td>
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<tr>
<td></td>
<td></td>
<td>M/L stiffness during Ad/Ab rotation</td>
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</tbody>
</table>

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<tr>
<th>3. Split Linearized</th>
<th>Proportion of Variance</th>
<th>Top 3 Contributors*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC1</td>
<td>28%</td>
<td>A/P stiffness during Anterior translation</td>
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<tr>
<td></td>
<td></td>
<td>M/L stiffness during Medial translation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ad/Ab stiffness during Medial translation</td>
</tr>
<tr>
<td>PC2</td>
<td>22%</td>
<td>F/E stiffness during Extension rotation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C/D stiffness during Adduction rotation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>F/E stiffness during Adduction rotation</td>
</tr>
<tr>
<td>PC3</td>
<td>13%</td>
<td>Ad/Ab stiffness during Extension rotation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M/L stiffness during Extension rotation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M/L stiffness during Adduction rotation</td>
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<table>
<thead>
<tr>
<th>4. Averaged Split Linearized</th>
<th>Proportion of Variance</th>
<th>Top 3 Contributors*</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC1</td>
<td>31%</td>
<td>M/L main</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ad/Ab stiffness during M/L translation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A/P main</td>
</tr>
<tr>
<td>PC2</td>
<td>21%</td>
<td>F/E stiffness during I/E rotation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>M/L stiffness during A/P translation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C/D stiffness during I/E rotation</td>
</tr>
<tr>
<td>PC3</td>
<td>16%</td>
<td>F/E main</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A/P stiffness during C/D translation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>A/P stiffness during Ad/Ab rotation</td>
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*Note: For example only, PC interpretations were made based on entire set of up to 22 significant (p<0.05) terms.

Table 6.3. Summary of PCA results for the four stiffness analysis techniques employing linear estimation of hysteresis curves. Main translational stiffness terms are present in each of the primary principle components. Interactions involving the M/L, C/D, and F/E DOFs are present in secondary PCs. Most of the Ad/Ab interactions are found in the tertiary PCs. PC1 and PC2 are more difficult to interpret as they only account for 10-25% of the total variation present in the entire data set.

Results of the spline modeled stiffness data revealed similar proportions for each of the 3 PCs (33%, 20%, and 16%) but with slight differences in the interpretations. The primary PC represented cross-coupled terms of A/P and Ad/Ab stiffness during M/L translation, the secondary PC still represented transverse and sagittal plane interactions, and the tertiary PC still represented Ad/Ab stiffness in addition to I/E stiffness cross-coupled terms.

Predictive Modeling

Models generated using only the two geometric PC factors did not adequately predict variation in gait dynamics PCs and did not identify either geometric PC as significant. Models generated using only stiffness factors showed the best results using only factors from the spline modeling technique. This model accurately predicts G1 (R=0.902, p<0.001) with s1 of the spline stiffness model beta coefficients as the significant factor (p<0.001). The next best model generated using only stiffness factors from the averaged split linearized technique identified s2 and s3 as
significant (p<0.05) in predicting G1 (R=0.716, p<0.05). Models combining geometric and stiffness PCs as factors produced the best results when only spline modeled stiffness PCs were used to predict G1 (R=0.879, p<0.05). However, none of geometric or stiffness factors were determined to be statistically significant in this combined model.

Models generated using the “leaps” function again were most statistically significant when using spline modeled stiffness factors compared to other techniques. G1 was accurately modeled (R=0.915, p<0.0001) by PC1 of spline modeled stiffness beta coefficients (p<0.0001). G2 was modeled (R=0.648, p<0.05) by PC2 of spline modeled stiffness beta coefficients (p<0.01). G3 was modeled (R=-0.621, p=0.099) by PC3 of spline stiffness (p<0.01). Models using split linearized stiffness factors showed G1 modeled by split stiffness PC2 (R=0.662, p<0.05, term significance p<0.01), G2 modeled by split stiffness PCs 2 and 3 and geometry PC1 (R=0.680, p=0.06, term significance p<0.05), and G3 modeled by split stiffness PC1 and geometry PC1 (R=0.636, p=0.05, term significance p<0.01). All other stiffness techniques show stiffness PC2 (s2) as a predictor of G1, and linear and averaged split linearized also identified s1 and g1 as predictors of G3.
Table 6.4. Summary of linear modeling results including results from spline modeled stiffness and each of the four stiffness analysis techniques employing linear estimation of hysteresis curves. The spline technique allowed more variability to be modeled but does not include main stiffness terms. This may explain why significant factors differed from the rest of the stiffness techniques.
Table 6.5. Combined summary of PCA interpretations. Stiffness PCs shown are most similar to linearized stiffness estimation techniques, as the spline technique did not incorporate main stiffness effects. Main stiffness effects had high contributions to the primary PC of each of the linearized stiffness PCA analyses.

Discussion

Results of geometric PCA on anatomic features align with previous research correlating anatomic differences with recorded kinematics. Smoger et. al simulated squatting in a cadaveric model and correlated tibio-femoral shape modes to resulting kinematic modes. The first shape mode described uniform scaling while the second described the J-curve of the condylar radius\textsuperscript{119}. This is analogous to geometric PC1 (scale factor) and PC2 (notch height). Mode 1 accounted for the majority of variation in initial kinematic alignments, ad/abduction rotations, and joined with mode 2 to explain variability in sagittal translations. Similarly, this study found geometric PC1 (scale factor) is a predictor of average A/P forces in the sagittal plane and associated torques.

PCA has been shown to be an effective method of analyzing population variability within orthopaedic research. A review by Bischoff et. al. has shown consistent findings among studies of anatomic variability\textsuperscript{113}, which includes the primary geometrical mode as a scale factor. While these previous studies have only investigate anatomic impacts on kinematic variability, this study adds to the literature by providing insight into the resulting dynamic loading patterns.
By simulating in vivo kinematics in a repeatable in vitro environment, we have shown that kinematic deviations do translate to deviations in functional requirements for knee structures.

Overall results of this study demonstrate that while anatomic geometry can be utilized as a successful method of defining population variability within the knee joint, it does not directly predict joint dynamics during simulated in vivo motion. For prediction purposes, joint stiffness metrics were much more powerful.

In interpreting the resulting generalized linear models, the first conclusion is that topography of the joint articulation (PC2 of linearized stiffness terms) is related to compression values during load bearing segments of activity (PC1 of gait dynamics). This is intuitive as uneven surfaces will generate much more variable normal loading profiles when articulated and moved with respect to another surface. While the spline modeled non-linear stiffness terms showed a slightly different result, this technique was most difficult to interpret as none of the main stiffness affects were considered in the data set. Although joint topography is more of a geometric attribute of the anatomy, none of the calculated geometries encompassed this phenomenon, leaving stiffness terms cross-coupled with compressive/distractive stiffness the most representative of uneven articulations.

The second conclusion is that Ad/Abduction stiffness (PC3 of linearized stiffness terms) can determine medial/lateral joint dynamics with its associated torques for simulated in vivo gait (PC2 of gait dynamics). Ad/Ab stiffness can be considered the best approximation to overall joint stiffness as geometric features have the least contribution to load distribution. Variability can primarily be attributed to bone and ligament material properties. Therefore, it is fitting that this type of stiffness is related to medial/lateral force and associated ad/abduction and
internal/external torques, as these values are primarily driven by material properties rather than geometric features.

The third conclusion is that translational laxity, including main stiffness contributors (PC1 of linearized stiffness terms), along with a joint size scale factor (PC1 of geometric stiffness) is loosely predictive of anterior/posterior dynamics with associated torques during gait. This result has much to do with the envelope of laxity within the joint. The larger and looser the joint, the less load will be observed in non-compressive DOFs during motion. As has been noted in previous studies in our lab\textsuperscript{120}, larger limbs generate less anterior force during heel strike and drive phases of stance because they can accommodate larger ranges of motion, particularly anterior motion, as the knee extends. This is a consequence of using only 1 standardized motion to test an entire sample of the population.

These findings have tangible implications for test method development using controlled kinematics to measure in situ loading patterns. For example, smaller specimens likely require a scale factor to the anterior/posterior kinematic DOF so as not to over translate the joint past a physiologically relevant position. This strategy has already been employed in our lab to dampen skin marker errors in recorded athletic activities using bone pin data to define the scale factor\textsuperscript{121}. This method enabled the successful simulation of side step cutting and drop vertical jump for 12 unique specimens without damage to joint structures or articulations. Models developed in this study may be used to develop tailored gait kinematics, according to the same recorded geometries and stiffness attributes, with the goal of recording more consistent and representative joint dynamics for in vitro simulations of in vivo motion. This will be critical moving forward in orthopaedic research where physiologically relevant test methodologies are in high demand.
Clinically, this study has reaffirmed and value of joint laxity tests to help assess functional deficits as well as to characterize demands specific to individual patients. Laxity tests such as anterior drawer and Lachman’s test are already widely used to assess the function of the ACL, whose primary role is to resist anterior translation. While these tests are typically done to diagnose injury, they may also be useful in establishing design criteria for reconstructions which are customized to the patient’s individual joint biomechanics.

The current study has several limitations. First, PCA was only able to capture between 63% and 82% of the variability between specimens, with stiffness properties being the most difficult to summarize. Representation of variability is further reduced in the predictive models when applying a linear fit. Models predicting the 2nd and 3rd PCs of gait dynamics only accounted for up to about 40% of the variability between specimens. While these levels of cumulative proportion may be insufficient for numerically accurate predictive modeling, relationships between factors (geometries and stiffness) and responses (metrics of gait dynamics) are still valid, allowing us to identify the key contributors. A second limitation is potential measurement error. Geometric measurements were done manually with a CMM where landmarks were chosen by eye. Other studies have used CT and other scanning techniques to fully digitize the entire articulation surfaces, leading to much greater resolution in their statistical shape modeling techniques. While stiffness and gait dynamics were measured more repeatably with programmable robotics, variation can still come from slight inconsistencies between limb set-up and orientation. Although initial orientation was controlled to ±1°, this could be enough offset to impact load responses in some of the smaller specimens. However, according to a previous study of our methodologies, these small deviations between set-up positions do not lead to significant differences in resulting dynamics. Another important
limitation of this study is that while in vivo motion was simulated, the in vitro environment lacks certain properties of the in vivo environment which may affect biomechanical response. While all specimens were constantly hydrated using saline soaked gauze, testing was performed at room temperature and without the lubricity of the synovial fluid. This may have led to slightly higher loads during stiffness and gait testing compared to the true in vivo scenario.

Results of this study identify the unique patient attributes which impact the functional requirements of the knee and the importance of tailoring in vitro test methods to represent physiologic scenarios. Models established in this study may be further developed to tailor biomechanical test methods to individual specimens, thereby increasing our ability to model physiologic conditions and improving predictive capacity of in vitro screening for new and novel repair techniques.

Acknowledgements

The authors thank Matthew Haaga for assistance in specimen preparation. Research reported in this publication was supported by the National Institute of Arthritis and Musculoskeletal and Skin Diseases of the National Institutes of Health under Award Number AR056660.
Chapter 7

Tracking knee remodeling due to meniscus and MCL injury via vertical ground reaction force

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This manuscript is currently in preparation for the \textit{Journal of Biomechanical Engineering}

Abstract

\textbf{Purpose:} In many cases, patients undergoing reparative knee surgery sustain injury to more than one structure. Although reconstructive techniques are available for the most critical structures (typically cruciate ligaments and especially the ACL), there is limited understanding of how concomitant injuries to multiple knee structures interact to influence knee biomechanics on an organ system level. Therefore, this study aimed to determine the extent to which two of the most commonly injured structures affect knee function by measuring changes in gait pattern. \textbf{Methods:} Four groups of normal, medial collateral ligament (MCL) injured, medial meniscus (MM) injured, and dual MCL and MM injured sheep were a part of this prospective study. Vertical ground reaction forces (VGRFs) were recorded at one speed (1 m/s) during level (0\degree), inclined (+6\degree), and declined (-6\degree) walking conditions using an instrumented treadmill with two force plates to measure
fore and hind limb VGRFs. MCL injuries were imposed by removing a 1 cm section of the ligament spanning the joint line. MM injuries were imposed by removing the MM in its entirety at the anterior and posterior horns. Post-injury hind limb VGRF data were recorded twice weekly for a period of 12 weeks during which the animals were allowed to move freely. **Results:** Throughout the study, MCL-injured VGRFs were parity or slightly increased compared to pre-injury levels during early stages of stance. MM- and dual-injured knees experienced a significant initial decrease with a slow increase back toward normal until there was no statistical difference from pre-injury values during level and inclined gait. However, during declined gait, VGRFs near the end of MM-injured stance increased to higher than pre-injury levels. Data also showed that VGRFs of the non-injured limb may also be affected by MM and dual injuries. VGRF changes leveled off at approximately 8 weeks. These findings suggest that patients sustaining multiple injuries including the MCL or MM may require specialized reconstruction techniques to mitigate affects, and that VGRFs may be a useful metric for indirectly assessing knee function in vivo.

**Introduction**

Injury of the knee is a common occurrence in the US, with anterior cruciate ligament (ACL) injury having some of the most severe consequences. ACL injury is a significant socioeconomic problem affecting 1 in every 3000 people annually with an estimated cost of $17,000 per reconstruction\(^{15,33}\). The loss of this restraint disrupts the natural joint kinematics and loading patterns experienced by surrounding knee structures. Over time, these altered knee dynamics can lead to joint degeneration and early onset of osteoarthritis (OA), a painful degenerative joint disease\(^{5,122-127}\). Strategies to repair the injured ACL show short term promise in restoring knee function, yet current reconstruction techniques continue to fail in the long term at rates of up to 27%\(^{128-133}\). Furthermore, reconstruction does not prevent the early onset of OA\(^{80}\). These short-
comings indicate that current repair strategies do not adequately restore the function of the healthy ACL. Therefore, the overarching goal of our research lab is to establish functional tissue engineering parameters under physiologic conditions to provide evaluation benchmarks and design criteria for ACL repairs.

However, these functional demands may differ in patients experiencing dual injuries to surrounding knee structures. Techniques for treating concomitant injuries to the ACL and surrounding structures remains widely debated throughout the field. Approximately 13% of patients with an ACL injury also sustain a dual medial meniscus injury, and about 30% sustain a dual medial collateral ligament (MCL) injury. Both structures are frequently left unrepaired, which can result in a wider range of knee motion and potentially greater forces applied to the ACL. However, in vivo loads on the ACL have yet to be accurately quantified in the injured population, leaving a significant gap in research for treatment of patients with dual injuries.

The MCL and medial meniscus have been shown to resist passive varus-valgus and internal-external rotation, indicating that their absence may influence ACL function. However, these cadaver studies cannot account for any biological significance that these structures may have on joint function. Studies have shown that mechanoreceptors reside in both MM and MCL tissue which may influence proprioceptive pathways and neuromuscular function. Furthermore, these resulting joint biomechanics could continue to change as structures heal and remodel in response to the injury. Both of these factors may contribute to changes in the control scheme of the knee itself, impacting physiologic kinematics and/or load response.

Laboratory evaluations during in situ simulations are not sufficient to capture these affects. Even in vivo studies are limited by the invasive nature of sensing probes that must be implanted to measure loading and/or kinematic patterns and the cost of more recent advancements.
fluoroscopic techniques. Therefore, this study presents a non-invasive in vivo approach using vertical ground reaction forces (VGRFs) to detect biomechanical changes during activities of daily living (ADLs) and tracking the knee’s recovery from MCL transection, medial meniscectomy, and a combined MCL transection and medial meniscectomy. VGRFs in both healthy and injured hind limbs will be compared to identify differences in knee function which could impact knee reconstruction and/or ACL repair requirements. Results of this study will improve current requirements and expectations for repair constructs in patients with dual injuries and approximate the time after injury needed for the knee to fully stabilize. Furthermore, these VGRFs will serve as in vivo metrics unaltered by additional sensing equipment as part of a larger study designed to quantify in vivo loading patterns via robotic simulation.

**Methods**

Twenty-seven (27) skeletally mature, female Suffolk sheep (age: 2-6 yrs; weight: 100-200 lbs) were used in this IACUC approved study. Four groups of normal (N=8), MCL-injured (N=6), MM-injured (N=6), and dual-injured sheep (N=7) were walked twice weekly at 1m/s on an instrumented treadmill (Kistler Gaitway) to collect VGRFs during 3 ADLs: level (0°), inclined (+6°), and declined (-6°) gait. To minimize fatigue and training effects, grade conditions were randomly chosen and data collected. After a 3-week training period, injured groups underwent surgical removal of the MCL and/or the medial meniscus. Injuries were performed by an orthopaedic surgeon (one of the following co-authors: M. Galloway, S. Harms, or M. Taylor) who exposed the medial aspect of the left hind limb to gain access to the knee. MCL injuries were imposed by removing a 1 cm section of the ligament spanning the joint line. MM injuries were imposed by removing the MM in its entirety at the anterior and posterior horns. Post-injury VGRF
collection resumed for a period of 12 weeks along with the normal group, during which the animals were allowed to move freely.

Representative VGRFs for each injury group, time point, and ADL were calculated for each animal by normalizing to body weight (%BW) over a full gait cycle and averaging using at least 5 consecutive strides. Two-sample t-tests compared average VGRF values between groups along with values at 30 and 38% of gait. One-way ANOVAs were used to examine 1) the influence of recovery time on VGRFs for pooled ADLs, 2) the influence of injury on VGRFs during each separate ADL and 2) the influence of ADL on VGRFs from each injury for both pre-injury and 12-week recovered time points at selected points of the gait cycle: injured hoof strike (approximately 8% of the gait cycle), and the beginning (22%) and end (42%) of injured hind limb stance. Paired t-test were then performed on pooled and individual ADL data sets to determine statistical significance of differences between pre-injury and 12 week time points at each of the selected gait cycle points.

Results

Pre-injury VGRFs were similar across animals of all groups during level gait (Figure 7.1), with MM and MCL groups only demonstrating slight differences during inclined (Figure 7.2) and declined (Figure 7.3) gait, respectively. However, according to two sample t-tests, results of these instances were not statistically different between any two samples. 12-week recovery VRGRs showed the greatest change at the beginning and end of stance, with inclined gait producing the most differentiation between groups. Inclined gait of Normal and MCL groups produced mid stance VGRFs that were each significantly higher than MM and Dual groups after 12 weeks of recovery (p<0.05, Figure 7.2.).
Figure 7.1. Level gait VGRFs of pre-injury and 12 week recovery time points (SEM bars) highlighting A) injured hoof strike (approximately 8% of the gait cycle), B) the beginning (22%) and C) the end (42%) of injured hind limb stance. Pre-injury comparisons show no gross differences in gait patterns across groups. Recovery VGRFs are also similar across groups with MM and Dual groups dropping slightly at B.
Figure 7.2. Inclined gait VGRFs of pre-injury and 12 week recovery time points (SEM bars) highlighting A) injured hoof strike (approximately 8% of the gait cycle), B) the beginning (22%) and C) the end (42%) of injured hind limb stance. Pre-injury comparisons show that the MCL group experienced larger VGRFs during single limb stance compared to all other groups.

Recovery VGRFs show further differentiation in amounts of reduction during stance.
Figure 7.3. Declined gait VGRFs of pre-injury and 12 week recovery time points (SEM bars) highlighting A) injured hoof strike (approximately 8% of the gait cycle), B) the beginning (22%) and C) the end (42%) of injured hind limb stance. Pre-injury comparisons show that the MM group experienced lower VGRFs during single limb stance compared to all other groups. Recovery VGRFs show less differentiation.

VGRFs of the MM and Dual injured hind limbs both experienced sharp drops just after the injury was created lasting around 2 weeks (Figure 7.4.). VGRFs then continued to climb until leveling off between 6 and 8 weeks. Three ANOVAs performed for each point of interest during gait revealed that time very significantly influenced VGRFs of all three points of interest.
in the MM group (p<.001) and peak load VGRFs in the Dual group (p<.001), and also significantly influenced beginning of stance VGRFS in the Dual group (p<.05, Figure 7.4). However, paired t-tests comparing VGRFs after 12 weeks of recovery to pre-injury values showed that the Dual group remained significantly different for all points of interest while the MM group only remained changed for VGRFs at the beginning of stance (p<.05, Figure 7.4). The MCL group also approached significance when comparing pre-injury to 12 week values of beginning of stance VGRFs (p=0.057).

VGRFs of Normal and MCL groups did not significantly change from pre-injury values and time was not shown to influence any point of interest during the gait cycle (Figure 7.4).

Breaking VGRFs into pre-injury and 12 week recovery values for each ADLs, one-way ANOVAs were performed to evaluate the influences of both injury and ADL (level, inclined, or declined gait).

Pre-injury peak loads were not influenced by injury group during any ADL, meaning all injury groups demonstrated similar VGRFs before injuries were imposed. Pre-injury peak loads were significantly influenced by ADL in the Dual group (p<.05), with the Normal group also approaching significance (p=0.056, Figure 7.5). Recovery peak loads were also unaffected by injury group while ADL was most influential in the Normal group (p<0.05) with the Dual group also approaching significance (p=0.07, Figure 7.5).
Figure 7.4. Overall VGRF changes over time with ADLs pooled. ANOVA showed VGRFs were most influenced by time in the MM and Dual groups. The dual group also showed sustained significant differences at 12 weeks compared to pre-injury (week 0) levels at each portion of gait according to a paired t-test. The MM group also showed sustained significant differences in VGRFs from pre-injury at the beginning of stance.
Figure 7.5. ANOVA results at peak load during gait. Pre-injury peak loads were not influenced by injury group during any ADL but were influenced by ADL in the dual group with the normal group also approaching significance. Recovery peak loads were also unaffected by injury group, while ADL was most influential in the Normal group with the Dual group also approaching significance.

Pre-injury VGRFs at the beginning of stance were unaffected by injury group, though it did approach significance during inclined gait (p=0.051). ADL was influential in pre-injury VGRFs of all groups (p<.05), especially in Normal and MCL groups (p<.001, Figure 7.6). Recovery VGRFs at the beginning of stance were influenced by injury for all ADLs (p<0.05), especially level gait (p<.001), and were also influenced by ADL in all groups (p<0.05), especially MCL and Dual groups (p<0.001, Figure 7.6).
Figure 7.6. ANOVA results at the beginning of stance. Pre-injury VGRFs at the beginning of stance were unaffected by injury group while ADL was influential in all groups. Recovery VGRFs at the beginning of stance were influenced by injury for all ADLs, and were also influenced by ADL in all groups.

Pre-injury VGRFs at the end of stance were also unaffected by injury group, while ADL was influential in the MCL group (p<0.05) and especially in Normal, MM, and Dual groups (p<0.001, Figure 7.7). Recovery VGRFs at the end of stance were, again, not influenced by injury for any ADL, but were influenced by ADL in the MM group (p<0.05) and especially Normal, MCL, and Dual groups (p<0.001, Figure 7.7).
Figure 7.7. ANOVA results at the end of stance. Pre-injury VGRFs at the end of stance were unaffected by injury group while ADL was influential in all groups. Recovery VGRFs at the end of stance were not influenced by injury for any ADL but were influenced by ADL in all groups.

Further breaking down the results into each specific ADL and strictly examining changes from pre-injury to post-recovery values, paired t-tests were performed to evaluate changes within separate ADLs for each group.

Comparing level gait VGRFs, beginning of stance values significantly dropped for all three injury groups (p<0.05), especially the Dual group (p<0.001, Figure 7.8). Peak load VGRFs approached significance when comparing the drop between pre-injury and recovery values of the Dual group (p=0.095, Figure 7.8). No significant changes occurred at the end of stance.
Figure 7.8. 12 week differences during level gait showed a drop in VGRFs at the beginning of stance for all three injury groups. No significant differences were observed at peak loading or end of stance.

Comparing inclined gait VGRFs, beginning of stance values significantly dropped in the MM group (p<0.001) and approached a significant drop in the MCL group (p=0.069, Figure 7.9). End of stance VGRFs significantly also dropped in the MM group (p<0.05) and approached significance when comparing the drop between pre-injury and recovery values of the Dual group (p=0.086, Figure 7.9). No significant changes occurred at peak load.
Figure 7.9. 12 week differences during inclined gait showed a drop in VGRFs at the beginning and end of stance for the MM group. No significant differences were observed at peak loading or for any other injury group.

Comparing declined gait VGRFs, beginning of stance values significantly dropped in the MM group (p<0.05, Figure 7.10). No other significant changes occurred at any point in gait for any group, though an increase in end of stance VGRFs did also approach significance for the MM group (p=0.094, Figure 7.10).
Figure 7.10. 12 week differences during declined gait showed a drop in VGRFs at the beginning and end of stance for the MM group. No significant differences were observed at peak loading or for any other injury group.

Discussion

Recovery time most significantly influenced the MM group followed by the Dual group (Figure 7.4). This is likely due to the associated trauma of extracting the MM without damaging the MCL, which requires high levels of abduction torque to be applied to the knee during the injury surgery. The Dual injury surgery is the second most traumatic, which explains the drops in injured limb VGRFs during the first 2 weeks of recovery for these groups. This corresponds to the period of time when gait was most affected by limping. Limping had typically subsided by week 3, and VGRFs of the MM and Dual groups began rising back up to pre-injury levels, with improvements
leveling off between 6 and 8 weeks. This plateau may indicate the time needed for the stifle joint to achieve complete healing with along with any remodeling which may have occurred.

Results of paired t-tests comparing pooled ADL pre-injury VGRFs to 12 week recovery values suggest that the Dual-injured knees will more permanently sustain more statistically significant changes to gait patterns in the long term compared to either single MCL- or MM-injured knees. This is indicated by the recovery value of all three points of interest (peak load, beginning, and end of stance) testing as different from the pre-injury value of the pooled ADLs (Figure 7.4). However, when VGRFs are analyzed separately for each level, inclined, and declined ADL, MM-injured knees sustain more statistically significant changes, especially during the beginning of stance (Figures 7.8-7.10). This is also reflected in the pooled ADL test, as the MM group shows significantly different values from pre-injury at the beginning of stance, only (Figure 7.4). While testing also showed differences in MM VGRFs at the end of stance during level gait, declined and inclined differences were not enough to make the pooled test significant. In contrast, although Dual VGRFs were statistically different at all points of interest in the pooled test, significance was spread more evenly across ADLs, leaving VGRFs at the beginning of stance during level walking the only statistically significant change once ADLs were broken out separately.

In investigating the influences of injury and ADL, results showed injury did not influence peak load VGRFs, while ADL did influence peak load VGRFs in Normal and Dual groups, only (Figure 7.5). However, this may have been due to a sampling artifact as these groups contain the most animals. A larger study would be needed to assess peak value differences as this point during gait is subject to the highest levels of variability. Both injury (for recovered animals) and ADL (for pre-injury and recovered animals) influenced beginning of stance VGRFs (Figure 7.6), while only ADL influenced end of stance VGRFs for both pre-injury and recovered time points (Figure
These results indicate that the beginning of single limb stance is the most susceptible to be altered due to injury. This is analogous to the time point that the animal begins to push itself forward, a critical time for knee function and biomechanics.

As such, declined gait showed the least change between pre-injury and recovery conditions. This may be due to the role that gravity plays in this ADL, which reduces the animals’ dependence on the musculoskeletal system to propel itself. This explains the lack of resolution between injury types. However, by this logic, the inclined ADL should have shown the greatest resolution between injuries, while the data showed that level gait demonstrated the most change in gait pattern. It is possible that inclined gait did not show as many statistically significant because of increased intra-specimen variability, as it was a more difficult task for the animals to complete.

For all activities, the MCL group demonstrated the least amount of change throughout recovery while animals without a MM (both MM and Dual groups) experienced the most change. While these changes are characterized as drops in hind limb VGRFs, the fore-limbs did not take on any additional weight (data not shown), eliminating the possibility that the animal was able to shift its weight to the front. One possible explanation for this isolated hind limb drop in force may be an internal compensation mechanism which alters kinematics through the proprioception pathways of the knee. All three structures, ACL, MCL, and meniscus have been shown to contain mechanoreceptors which directly tie into the central nervous system to affect proprioception and can alter motion patterns\textsuperscript{137-139}. While little is known about these pathways, it is possible that they function to avoid harmful joint biomechanics. Changes to the Dual group resided in between the MCL and MM groups, supporting that such a compensation mechanism has an additive effect on joint biomechanics. However, variability was too high to establish statistical differences between each of the three injury groups.
Limitations of this study include relatively high intra- and inter-subject variability during recovery leading to low statistical power. For those reported differences only approaching statistical significance, the calculated power values stayed below 0.5. Still, results are able to shape hypotheses of future studies interested in in vivo biomechanics of patients with MM, MCL, or Dual injuries. Beyond the unique gait patterns specific to individual subjects, variability may have also been introduced by slight deviations in surgical techniques due to varying anatomy in addition to subject healing response. While no subject demonstrated visible signs of additional injury after their initial injury surgeries, animals were free to move about and could have induced additional trauma in the middle of the study. In some cases, animals with shorter torsos exhibited forelimb/hindlimb crossing, which interfered with hindlimb force plate measurements, leading to more challenging data collection. However, this was mitigated by increasing the data collected to assemble more pure hind limb strides.

Results of this study support the hypothesis that injury to surrounding structures induces changes to knee function during healing, which may ultimately impact demands on the ACL in a way that in vitro testing alone cannot evaluate. Results will aid in establishing treatment and diagnostic strategies for patients sustaining injury to the MCL and/or medial meniscus (MM), with the goals of: 1) Accelerating patient return to pre-injury activities of daily living and 2) slowing or stopping premature onset of osteoarthritis.

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Effects of medial meniscus and MCL injury and natural healing on knee joint dynamics and cartilage health in the sheep model.

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Abstract

Purpose: Our current understanding of anterior cruciate ligament (ACL) function is based on simulations of clinical examinations and on the application of non-physiologic load and motion patterns. These methodologies likely provide little information on ACL function during activities of daily living (ADLs). Due to the difficulties associated with in vivo sensing, researchers have yet to directly measure the physiologic strains or loading demands on the ACL. Furthermore, it is unknown the extent to which these demands are altered due to changes in activity or in the presence of additional knee pathology, as the medial collateral ligament (MCL) and medial meniscus (MM) are frequently injured in tandem with the ACL. This study aimed to quantify the effects of injury to these structures during varying activity levels on knee kinematics and corresponding joint and
ACL loading profiles during simulated in vivo motions. **Methods:** 28 female Suffolk sheep were divided into Normal, MCL injured, MM injured, and dual injured groups. After surgical injury, animals were walked twice weekly on a treadmill during level (0°), inclined (+6°), and declined (-6°) gait conditions over 12 weeks of healing. Electromagnetic (EM) tracking sensors were then surgically implanted onto the left distal femur and the left proximal tibia and in vivo motions were recorded post-implantation for all activities. Following sacrifice, each set of 6 degree-of-freedom (6 DOF) motions were applied to their respective knee joints using a serial robot instrumented with a multi-axis load cell. In vitro simulations were repeated to measure a) total knee loads, b) contact pressure maps, and c) ACL-only loads. Cartilage was then evaluated for signs of osteoarthritis (OA). **Results:** MM animals saw increased ACL forces during the transitions between swing and stance in response to significant increases in anterior translation. Dual animals saw no increases, yet both groups developed significant OA within the medial compartment. MCL animals saw increased ACL force during mid stance in response to increased overall joint laxity but no increase in OA. Results of the this study are the first to relate ADLs to resulting knee biomechanics and ACL function and provide preliminary data for defining design requirements for future ACL reconstruction techniques.

**Introduction**

After an ACL injury, the loss of ACL function dramatically alters knee kinematics, altering joint contact pressures and causing further health problems. Orthopaedic surgeons aim to restore natural kinematics to the knee. However, current ACL reconstructions are deemed successful purely based on the restoration of anterior tibial translation. Furthermore, there is no diagnostic difference used between patients with single ACL and dual MCL or meniscus injuries. Neglecting to assess repairs in all anatomical directions and ignoring the specialized needs of dual injury
patients may contribute to clinicians’ inability to provide ACL reconstructions that satisfy in vivo demands.

Because native ACL forces cannot be directly measured in vivo without physically altering the path of the ACL, investigators have attempted to measure the functional demands of the ACL by performing cadaveric studies. Previous studies have shown that its anatomic position and complex structure make the ACL a major stabilizer in restraining anterior tibial translation, internal rotation, and varus-valgus angulation\textsuperscript{1,43-48}. This diverse function is thought to be promoted by the existence of two distinct bundles, the anteromedial (AM) and posterolateral (PL), which twist and untwist around one another throughout the knee’s range of motion\textsuperscript{49}. Such complexity makes it essential to quantify native loads in all directions to fully describe functionality. Yet researchers typically are only able to study ACL force in a fixed knee position along a single loading direction\textsuperscript{51,52,54-61}. As a result, current ACL force data is rarely derived from actual in vivo conditions.

In addition to inherent complexity, the impact of surrounding knee structures on the ACL also plays a role in its poorly defined function. Approximately 13\% of patients with an ACL injury also sustain a concomitant medial meniscus (MM) injury, and about 30\% sustain a dual medial collateral ligament (MCL) injury\textsuperscript{62}. Both structures are frequently left unrepaired, though treatment of these concomitant injuries with the ACL remains a widely debated topic\textsuperscript{134}. Cadaver studies have shown that the MCL and medial meniscus resist passive varus-valgus and internal-external rotation, indicating that their absence may influence ACL function\textsuperscript{63-66}. Ma et al. found that MCL transection increased in situ ACL force by approximately 2.5 times when cadaveric knees were subjected to valgus torques\textsuperscript{65}. However, these studies were not performed under physiologic conditions and could not account for any biological significance these structures may
have on overall joint function after a period of healing. All three structures, ACL, MCL, and meniscus have all been shown to contain mechanoreceptors which directly tie into the central nervous system to affect proprioception and can alter motion patterns\textsuperscript{137-139}. Our group has shown that after removing these structures in a sheep knee, vertical ground reaction forces progressively lessen during single hoof stance over 12 weeks of healing, indicating that the knee (and perhaps the ACL itself) may remodel in response to the missing structures\textsuperscript{140}. Although these studies have provided valuable insight, in vivo loading of the ACL and influences of surrounding structures have yet to be sufficiently quantified.

This study is guided by the research question: How do functional demands for ACL reconstructions in patients sustaining concomitant injuries to the MCL and/or medial meniscus change compared to isolated ACL injuries? Due to the invasiveness of and inaccuracies caused by in vivo force sensing and motion capture in human subjects, this study makes use of the well-established sheep model to apply in vivo recorded motions to the in vitro specimens while recording loads. Sheep knees are similar to human in size and shape and previous studies in our lab have shown them to have similar biomechanics\textsuperscript{36,70}. Here, we investigate this question by imposing MCL, MM, and Dual injuries in sheep, recording in vivo kinematics, reproducing kinematics in vitro, and measuring corresponding loads across the knee and isolated ACL along. Results may then be compared to articular cartilage health.

This study presents a novel approach to overcome barriers in measuring ACL force by simulating motion data for multiple ADLs using robotic technology. Thus, in vivo motion may be reproduced in the laboratory to determine native ACL forces under more physiologic loading paths and environmental pathologies. ACL loads in both healthy and injured knees will be compared to identify differences in potential ACL repair requirements. These differences
represent the combined effects of kinematic changes due to structural loss and joint (including ACL) remodeling changes due to increased functional demands caused by injury to surrounding structures. Results of this study will improve current benchmarks for potential repair constructs in patients with dual injuries and potentially provide 3D loading profiles for simulating tissue engineered constructs grown in vitro. Furthermore, it will inform future in vivo studies aimed at testing the efficacy of potential ACL reconstruction techniques and provide a testing platform for evaluation of proposed reconstruction designs.

Lastly, this study goes beyond ACL characterization by examining the relationships between in vivo vertical ground reaction forces (VGRFs), kinematics, total knee loads, joint pressure maps, ACL loads, and cartilage degeneration. From these measurements, we were able to identify cause and effect relationships leading to joint degeneration following a knee injury. This information will aid in prioritizing ACL reconstruction designs that will mitigate deleterious effects to cartilage in patients with concomitant injuries.

**Methods**

*Experimental Design*

Twenty-seven (27) skeletally mature, female Suffolk sheep (age: 2-6 yrs; weight: 100-200 lbs) were used in this IACUC approved study. Four groups of 7 were initially split into normal, MCL-injured, MM-injured, and Dual-injured cohorts. However, due to complications with equipment, surgeries, and animal cooperation, the final data set contained 23 subjects consisting of 5 Normal, 6 MCL-, 5 MM-, and 7 Dual- injured animals. Methods of this study are identical to methods described in Chapter 3: “Effects of gait inclination on ACL and total knee forces during simulated in vivo motion in the sheep model” with the addition of injured animals. Animals of the Chapter 3 study were included in this study as the normal group. Injured animals of Chapter 7:
“Tracking knee remodeling due to meniscus and MCL injury via vertical ground reaction force” were included in this study within the injured groups.

Briefly, recall that after at least 3 weeks of animal training to walk on a treadmill, injuries were imposed surgically by one of the co-authors (Dr. Marc Galloway, Dr. Sam Harms, or Dr. Michal Taylor), described in more detail in Chapter 7, after which animals were allowed to ambulate freely. Following a 12 week period of healing (for injured groups), electromagnetic trackers are rigidly implanted to the tibia and femur of the left hind limb in each of the four groups as previously described in Chapter 3. Pre- and post-operative vertical ground reaction forces (VGRFs) were recorded to quantify the effects of tracker implantation during level (0°), inclined (+6°), and declined (-6°) gait conditions on an instrumented treadmill (Kistler Gaitway). Gait kinematics were collected at 2, 6, and 9 days to establish in vivo motion profiles for each group and for each of the three activities of daily living (ADLs). Following sacrifice, kinematics were reproduced on each respective specimen using a high-accuracy serial robot (KR210; Kuka Robotics Corp., Clinton Township, MI) while a six-axis load cell (Theta Model FT5498; ATI Industrial Automation, Apex, NC) and thin-film sensors (Tekscan) captured total knee and ligament loads and the joint pressure map between each condyle and the tibial plateau. Detailed photographs of the overall joint, each condyle, and each compartment of the tibial plateau were then evaluated by one of our surgeons (Dr. Marc Galloway) for assessment and regional scoring of the state of the articular cartilage using the Noyes Injury Classification System.

Data Analysis

All kinematic and load cell data were averaged over a single cycle and average and SEM values are reported here. Effects of ADLs were then evaluated using one-way ANOVA testing
with three levels of the test factor (surface inclination) to compare means of each ADL condition with respect to average value, maximum and minimum values, and range of each DOF. Data were then broken up into stance and swing phases and ANOVA tests were re-applied to each of these data sub-sets. Two-sample t-tests were then used to distinguish differences between injury groups for each metric. All statistically significant response measures were found to be normally distributed and homoscedastic. Responses that were not normal or homoscedastic were typically widely varying within the sample, leading to low power values and higher probabilities of type II error.

Results

VGRFs

As presented in the previous chapter, initial pre-injury VGRFs were not significantly different between groups, allowing direct comparisons of treatment conditions.

Comparisons between ADLs prior to implanting the EM trackers showed significant differences in average VGRF values between each grade condition across all injury groups (p<0.01, Figure 8.1. A). The only exception was a lack of difference between level and inclined gait conditions in the MM group. Post-implantation data showed similar trends, with significant differences between average values of all ADLs in the Normal and MCL group (p<0.01, Figure 8.1. B), between declined and inclined gait only in the MM group (p<0.01), and between declined and each remaining ADL in the Dual group (p<0.01). Comparisons between pre and post-implantation VGRFs show that while average VGRFs were statistically different for all ADLs across all groups (p< 0.05, Figure 8.1.) with the exception of the MCL group, relative trends between ADLs remained the same.
Comparing VGRFs between injury groups at 12 weeks of recovery, 12-week recovery VRGRs showed the greatest change at the beginning and end of stance, with inclined gait producing the most differentiation between groups. Inclined gait of Normal and MCL groups produced mid stance VGRFs that were each significantly higher than MM and Dual groups after 12 weeks of recovery (p<0.05, Figures 7.2. and 8.1.A.).
Figure 8.1. A. Average data for pre-implantation VGRFs for each ADL normalized to a gait cycle. Cycles begin and end with hoof strike and VGRFs are symmetric between right and left
limbs. B. Post-implantation VGRF data demonstrating altered gait patterns due to limping while maintaining the relative differences between ADLs. (SEM bars).

Kinematics

Results of ANOVA testing showed that inclination was a significant factor for posterior translation during push-off (p<0.05) and flexion angle at hoof strike (p<0.05) in Normal animals. The MCL group also showed inclination as a factor for these outcomes along with compression at push-off (p<0.05). Flexion angle at hoof strike (p<0.01), medial translation during push-off (p<0.05), and posterior translation during push-off (p<0.05) varied with inclination in MM animals. And flexion angle at hoof strike (p<0.05), posterior translation during push-off (p<0.05), and range of internal rotation during stance (p<0.05) varied with inclination in Dual animals.

Comparisons between groups showed that the MM group experienced greater anterior translation from hoof strike to mid stance (20% of gait) during stance compared to all other groups and during all ADLs (p<0.01). Dual groups during declined gait also greater anterior translation from hoof strike to mid stance compared to Normal (p<0.05, Figure 8.2.). The MM group also experienced a less posterior translation during swing compared to Dual animals for all 3 ADLs (p<0.05, Figure 8.2.). Compressive translations of the MM group at mid stance were also higher compared to all other groups during inclined gait (p<0.05) and also compared to MCL animals during declined gait (p<0.05, Figure 8.2.). The Dual group also experienced greater compression translation compared to the MCL group during declined gait, with statistics approaching significance (p=0.053). While Dual animals showed a trend of increased external rotation during stance this finding was not statistically significant due to sample variation. Dual animals and MCL animals showed less flexion rotation at hoof strike compared to MM and Dual animals in the
declined condition (p<0.05) and less flexion at push off compared to MM animals during level and declined gait (p<0.05) and Dual animals during declined gait only (p<0.01). During inclined gait, the range of flexion during stance was significantly lower in Dual animals compared to all other groups (p<0.05, Figure 8.2). Dual animals also demonstrated a smaller range of abduction during stance compared to MM animals during inclined gait (p<0.05), allowing these animals to remain more adducted throughout stance. Across all groups and conditions, A/P and C/D translations were directly proportional, as were I/E and Ad/Ab rotations.
**Level Gait Kinematics**

- Anterior (+) / Posterior (-)
- Medial (+) / Lateral (-)
- Compression (+) / Distraction (-)

**Inclined Gait Kinematics**

- Anterior (+) / Posterior (-)
- Medial (+) / Lateral (-)
- Compression (+) / Distraction (-)

---

% of Gait Cycle

Translation (mm)

Rotation (°)

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MCL
MM
Dual
Normal
Figure 8.2. Effects of Injury on Kinematics. Average in vivo knee kinematics with SEM bars for all ADLs: level (0°), inclined (+6°), and declined (-6°) gait. The gait cycle begins with hoof strike. Swing phase begins at approximately 62% of the gait cycle. Clear patterns are observable as gait is declined.

Intact Knee Loads

Inclination had the most significance for MCL animals, with anterior loads increasing with grade during at hoof strike (p<0.05) and medial loads increase with grade during push off (p<0.05, Figure 8.3.). While flexion and abduction torque at hoof strike in Dual animals also approached being significantly increasing with grade, inclination did not alter resulting knee loads as much as the kinematics. Across all conditions, A/P force and F/E torque was directly proportional while M/L force and Ad/Ab force were inversely proportional. C/D force and I/E torque were also directly proportional to a lesser extent.
Comparisons between groups showed that average anterior force during all ADLs was higher in MM animals compared with MCL animals (p<0.05) and was higher compared with Dual animals during inclined and declined gait only (p<0.05, Figure 8.3.). Average anterior force during declined stance was also higher for MM and Dual groups compared with both Normal and MCL groups (p<0.05) but were not significantly different from each other. Posterior forces and at push off of all ADLs were higher in MCL animals compared with both MM and Dual animals (p<0.05) which also resulted in lower flexion torques (p<0.05). Normal posterior push off forces during level and declined ADLs were also higher compared with MM and Dual groups (p<0.05). Compression forces during push off for Normal and MCL groups were each higher than both MM and Dual groups for all ADLs (p<0.05). Average compression force of the MM group was significantly lower than all other groups for all ADLs (p<0.05) and Dual animals showed less compression at hoof strike compared to MCL animals for level and declined conditions (p<0.05). The MM group experienced higher abduction torque compared to any other group, particularly during inclined gait (p<0.05).
Figure 8.3. Effects of Injury on Intact Knee Loads. Average intact knee loads during in vitro simulation with SEM bars for all ADLs: level (0°), inclined (+6°), and declined (-6°) gait. The gait cycle begins with hoof strike. Swing phase begins at approximately 62% of the gait cycle.

Moderate patterns are observable as gait is declined.

Pressure Maps

Contact pressures during mid-stance showed that MCL injured knees have greater pressure along the outer rim of the medial meniscus compared to normal knees. MM injured knees showed higher pressures over a larger, centralized area on the medial condyle. The Dual injured knee displayed the same medial condyle pattern as MM injured knees but with little to no pressure applied to the lateral condyle (Figure 8.4.).
Figure 8.4. Effects of injury on condylar pressure maps at Mid Stance. Representative pressure maps for A. Normal knees B. MCL injured knees C. MM injured knees and D. Dual injured knees. Mid Stance occurs at approximately 20% of the gait cycle.

**ACL Loads**

Inclination was approached significance for Normal animals, as anterior ACL force increase with grade at hoof strike (p=0.096) and distraction during stance also increased with grade (p=0.062). Inclination played the largest roll in MCL animals, with anterior, medial, and distraction forces along with flexion torque all increasing with inclination during hoof strike. (p<0.05, Figure 8.5.). The MCL group also showed higher flexion torques during peak swing
(p<0.05). In MM animals, while the values of anterior and flexion loads themselves were not significantly altered with grade, the ranges of anterior force and flexion torque both increased with grade during swing. Inclination in MM animals also approached significance as distraction force (p=0.095) and flexion torque (p=0.098) decreased during push off with increases in grade. In Dual animals, inclination approached significance for increases in both abduction torque (p=0.092) and anterior force (p=0.078) with grade during hoof strike.

Comparisons between groups showed that anterior force at hoof strike was greater in MM animals compared to all other groups during declined gait and also greater than Dual animals during inclined gait (p<0.05, Figure 8.5.). Peak anterior force during swing of inclined gait was higher in MM animals compared Dual animals (p<0.05) and approached significance compared to MCL animals (p=0.074, Figure 8.3.). The MCL group consistently showed increased anterior force throughout stance, especially when compared to Dual animals during inclined (p<0.05), level (p=0.067), and declined gait (p=0.068). MM animals showed greater distraction at hoof off compared with all other groups during declined gait (p<0.05) and the maximum distraction force was significantly great than the Dual group during the inclined condition (p<0.05). Average torques about the anterior axis of the knee showed that MCL and MM sheep differed the most during declined gait (p<0.05), with the MCL group producing adduction torque and the MM group producing abduction torque.
Level Gait ACL Loads

Inclined Gait ACL Loads
Figure 8.5. Effects of Injury on ACL Loads. Average ACL loads during in vitro simulation with SEM bars for all ADLs: level (0°), inclined (+6°), and declined (-6°) gait. The gait cycle begins with hoof strike. Swing phase begins at approximately 62% of the gait cycle. Moderate patterns are observable as gait is declined.

Articular Cartilage

After 12 weeks Normal and MCL animals developed minimal medial condyle cartilage damage (Figure 8.6.). MM and Dual groups developed significantly more areas of OA compared to both Normal and MCL animals (p<0.05, Table 8.1.). The area of Dual group OA was not significantly higher than that of the MM group, though degeneration appeared to be more severe in the anterior portions of the medial tibial plateau and medial femoral condyle (Figure 8.6.). Lateral compartments did not show any sign of degeneration in any group. A select group of
contralateral joints were also examined which revealed only one specimen presenting OA in the unaltered knee.

![Cartilage assessment matrix for each specimen included in the study according to the Noyes System.](image)

Table 8.1. Cartilage assessment matrix for each specimen included in the study according to the Noyes System. Normal and MCL groups showed negligible amounts of OA while MM and Dual groups showed central, mid, and peripheral degeneration on both the tibial and femoral surfaces.

No OA was observed in the lateral compartments. Only one specimen showed OA in the contralateral limb.
Figure 8.6. Representative samples of articular cartilage degeneration between groups.
Discussion

This study successfully examined the relationships between kinematics, total knee loads, joint contact pressures, ACL loads, and patterns of OA for animals with single and multiple knee injuries. As expected, animals lacking a medial meniscus in the MM and Dual groups experienced increased compression and accelerated development of OA in the medial compartment, although no significant increase in ACL load was observed. And while the small changes from normal in kinematic translation and joint pressure maps of the MCL group are consistent with previous literature, resulting kinematic rotations and increase in ACL loads are novel discoveries to the field.

Although passive rotation about the anterior/posterior axis has been proven lead to increased abduction in animals with MCL injury, active in vivo kinematic measurement showed that MCL-injured knees actually increase adduction during ADLs. These results suggest that animals are able to compensate for the loss of this structure by altering their normal gait kinematics. MCL-injured animals demonstrated increased adduction kinematics (Figure 8.2.), presumably to avoid large medial opening during activity. This phenomenon is also visible in the joint pressure maps where medial pressure increases along the periphery of the medial meniscus in MCL animals compared to Normal (Figure 8.4.). Joint contact pressure of Dual-injured animals also shifts more medially compared to the MM group. With the Dual injury group also comparing more closely to the MCL group in kinematics, data suggests that the meniscus plays less of a role in any potential kinematic feedback loop. One previous study reports that there is no measurable deficit in proprioception between ACLR patients with and without meniscus injury. Kinematic differences in the MM group were primarily seen in sagittal plane kinematics, indicating that menisci function more purely as mechanical restraints.
With up to 2 mm more anterior translation and up to 1.2 mm more compression at mid stance, MM animals demonstrated the greatest change in sagittal kinematics (Figure 8.2.). While MM animals produced the most anterior kinematics relative to hoof strike during stance, anterior ACL force did not follow suit beyond initial hoof strike. The greatest increase in ACL force throughout mid stance was observed in the MCL group. This is likely due to the increased compression translation of the MM group as a result of the missing meniscus, bringing the ends of the ACL closer together. This is supported given that while Dual and MCL groups maintained the most similar A/P and Ad/Ab kinematics (Figure 8.2.), MCL animals still produced more ACL force (Figure 8.5.). The lack of ACL loading in Dual may further be explained by a slight increase in external rotation (~2°, p>0.05) which would loosen the ACL within the joint and place more load onto the PCL. This external rotation explanation also aligns with the cartilage wear pattern of the dual animals, in which OA was more focused in the anterior portion of the medial femoral condyle (Figure 8.6.).

Total knee dynamics were the most difficult to interpret as the loss of various structures directly impacted loads transmitted. Therefore, insights about the MCL may only be made by comparing the MCL group (no MCL, meniscus) to Normal group (MCL, meniscus) or by comparing the Dual group (no MCL, no meniscus) to the MM group (MCL, no meniscus). Even then, confounding factors exist in the differing kinematics applied. Because of this, no strong conclusions surrounding injury could be made from total knee loads as part of this study design. The largest difference seen between groups was increased posterior and compression forces for Normal and MCL groups compared to both MM and Dual groups, which is attributable to the presence of the meniscus and its ability to transmit load. This is consistent with previous studies have shown that the bony interaction accounts for the vast majority of intact knee loads, followed
by the medial meniscus\textsuperscript{70}. However, even with the increased compression and adduction loads, MCL groups did not show any increase in OA progression. This highlights the importance of menisci to cartilage health.

As also presented in the results of Chapter 3 (investigating only the effects of ADL on normal biomechanics), the highest magnitudes of ACL loading were during swing phase. Combined with the observed lower ACL forces in the more compressed MM group, these results indicate that ACL loading is measured with the greatest resolution during less-compressed knee states. As mentioned in Chapter 3, while we may infer that ACL function is most critical during load bearing activity to prevent injury, there is no evidence of measurable increase in ACL demand during compression. One study compared ACL strain during active flexion with no resistance to ACL strain during squatting with body weight and found no difference\textsuperscript{85}. A more recent study showed that while the addition of external compression to the knee does not reduce strain in the ACL during passive flexion and extension, compression did shield the ACL from additional strain when resistance was introduced\textsuperscript{86}. This type of compressive shielding has also been demonstrated in previous studies of both cadaveric and sheep knee testing\textsuperscript{70}. Similar to how MM sheep showed less ACL force despite higher anterior translations, compression during stance may simply be bringing the origin and the insertion of the ligament closer together, thereby reducing the tensile load transmitted through the structure at these times.

Another possible explanation for low ACL loading during stance is artefact caused by “expansion” of the remaining bones after removal of the articulation to achieve the ACL-isolated condition during in vitro simulations. Without the femoral condyles present to properly fill the joint space between femoral origin and tibial insertion points, the remaining uncompressed bone may have been free to expand further into the joint space, bringing the two ends of the ligament
closer together and reducing overall tension on the ACL. While this would also affect loading during swing phase, this phenomenon coupled with compressive shielding could explain why minimal load was observed in the ACL during phases of each ADL when compressive load was highest.

These relatively low ACL loads may be a limitation of using the sheep model for robotic testing, as their knees have been shown to be tighter than the human knee joint\(^{36}\). This could accentuate the effects of compressive shielding and expansion artefact on measured isolated ligament loads. Previous studies have shown that while the human ACL is the third highest contributor to total intact knee loads (behind bony interaction and medial meniscus), the sheep ACL, measured by the same methods presented here, remains a relatively small contributor\(^{70}\). However, while ACL loading may be low, differences between injury conditions were still observable by the methods presented here, and current literature consistently supports the use of sheep as surrogate for ACL biomechanics, as it is critical to overall knee function.\(^{87,88}\)

Several other limitations existed within this study. First, relatively low sample size (the smallest was N=4 for ACL loads in the MCL group) and high variation led to low statistical power, leaving us unable to statistically detect some potential differences between groups during in vitro simulation. Power analyses determined that with the levels of variation observed in this study, a sample size of 10 or more would be more appropriate. It is also possible that some of the variation was due to kinematic error driven by EM tracking system interference, which could have influenced the ADL motions. While metal in the measurement field was kept to a minimum, the treadmill itself was enough to introduce error into measurements during pilot studies. To mitigate these effects, only relative tracker positions were used, so that this error would cancel out assuming both trackers were equally affected by the interference. Still, residual error from this process and
error from compounded measurements and calculations had the potential to stack up and significantly impact motions. Another consideration is the impact of motion tracker implantation on in vivo gait. While animals did demonstrate a visible limp during post-implantation gait, comparisons of pre-op to post-op VGRFs showed little relative differences between groups and ADLs. However, MCL animals did demonstrate noticeably less limp, as is indicated by a trend in higher VGRFs during left (injured) limb contact compared to other groups (Figure 8.1.). This could be an alternative explanation for increased ACL loads in MCL animals, as this group seemed to handle the implantation surgery better than the other groups. With further regard to in vitro test methods, gaining access to the joint to insert the Tekscan sensors by removing of collateral ligaments and the PCL could have had a small influence on the dynamics between the menisci and the tibial plateau, as the MM anchors onto the MCL.

Another limitation may be surrounding ad/abduction torque measurement, which is highlighted by the consideration that while pressure maps showed primarily medial loading for Dual animals without a meniscus, no adduction torque was measured during testing. This would have stressed the LCL resulting in adduction torque without an MCL to even it out the other way. In addition, no increase in adduction torque was observed in MCL animals, who showed increased adduction kinematics. It could be that gait does not produce ad/abduction torques high enough to be measured with enough resolution to detect differences between groups. It is also possible that the measurement of adduction torque was confounded by the placement of the joint center point (peak of the medial spine), about which all effective loads were calculated. Small variations in placement or relative motion between this point and the load cell (bone bending) could have resulted in amplified variation in ad/abduction torque calculation.
Despite these limitations, this methodology has proven effective in demonstrating differences in functional ACL demand between varying injury groups throughout multiple ADLs. Results showed that, while varying degrees of injury produces significant biomechanical differences throughout the gait cycle, especially in the sagittal plane, differences in ACL loading are less observable due to compressive shielding during stages of ADLs when higher proportions of body weight are applied to the joint. Kinematic factors increasing resulting ACL loading patterns included anterior and distraction translations and could be countered by compressive translation and external rotation. Thus, the MM injury group showed the greatest challenge to the ACL during hoof strike (0% gait) and hoof off (62% gait) during the transition between distracted and compressed knee states, while the MCL injury showed the greatest challenge to the ACL during mid stance (10%-40% gait). This finding challenges the notion that ACL loading is strongly linked to ground reaction force, a common input to computational ACL models, which has implications for designing future ACL replacement techniques. According to these results, the greatest potential for activity-driven functional deviation lies within the transition periods between unloaded and loaded knee states. This is consistent with many theories surrounding ACL injury prevention.

From a clinical perspective, patients sustaining MCL injuries may be more likely to see increased ACL loading during load bearing activities, as increased joint laxity may impact anterior and adduction kinematics. However, no increase in OA progression is expected. In fact, previous studies have shown little benefit in repairing the MCL in ACL reconstruction cases by current evaluation standards, though increased anterior and abduction laxity may persist. Alternatively, patients sustaining MM injury or meniscectomies are more likely to see increased ACL loading during transitions from distracted to compressed states, as load bearing activities my
induce compression shielding of the ACL. These patients may expect to develop OA within the medial compartment of the knee, with both the tibial plateau and the central-to-anterior regions of the femoral condyle affected. Patients sustaining both injuries may not see any increase in ACL loading during any activity, as external rotation loosens the ligament during transitions and compression shielding loosens the ligament during load bearing stages of activity. However, these patients may expect to develop similar levels of OA as compared to MM patients, with just slightly more risk to the anterior region of the medial femoral condyle.

Therefore, the circumstance under which it may be most beneficial to adjust the design criteria of a prospective ACL reconstruction would be for the MCL-injured patient. In this case, the potential graft material strength may be increased to sustain the additional loads of up to 5% body weight required for proper joint biomechanics, which have been shown here to resist early onset OA. However, if natural healing of the MCL is allowed before ACL reconstruction, as is practiced among some orthopaedic groups \textsuperscript{143}, this may be sufficient. Comparisons between MM and Dual outcomes suggest that there are no ACL graft modifications that can be done to prevent OA. Dual kinematics were both more posterior and more externally rotated, both routes by which the ACL could possibly provide a kinematic benefit. However, neither of these DOFs were able to prevent cartilage degeneration in Dual animals. Still, patients suffering from MM injury may also benefit from enhanced ACL reconstruction techniques preventing excessive anterior motion, as these patients are more susceptible to increased ACL loads during transitions from distracted and compressed knee states. While native ACLs have been shown to sustain up to approximately 150\% of a typical body weight (180 lbs)\textsuperscript{144}, traditional grafts and fixation methods are less reliable and may not always be sufficient to bear the increased load of up to 10\% body weight, potentially resulting in multiple surgeries to repair failed attempts.
This study contributes to a more thorough understanding of knee biomechanics and testing strategies as they relate to ACL function, and provides preliminary data for defining design requirements for future ACL reconstruction techniques. While ACL repair techniques may not be able to mitigate OA progression in patients sustaining concomitant injuries to the MCL and/or MM, the correlation between increased compression and OA shows that there is a clear risk of over-tensioning a graft which may actually accelerate degeneration. Future work will focus on more athletic activities in both cadaveric and animal models and will aim to better understand how to transition safely between uncompressed and compressed knee states. This robotic testing platform will continue to be utilized to examine many more factors governing ACL function and repair, with the goal of informing better ACL reconstructions to slow or stop the early onset of OA.

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Chapter 9

Discussion and Conclusions

This body of work has built on previously established characteristics of the ACL to further identify risks in patient activity and concomitant knee injury. These studies have shown that ACL loads increase during transitions between weight bearing phases of activity and that compressive shielding of the ligament occurs during the sustained compression of stance phase. Because of this and the lack of OA observed in MCL animals, stance-phase knee biomechanics and OA prognosis of patients undergoing meniscectomy or lacking MCL function may not be improvable by enhancements to ACL reconstruction techniques alone. However, these reconstructions can be expected to endure an increase in functional loading of between 5% and 10% body weight, which is an important consideration to avoid ACL graft rupture in patients sustaining concomitant injuries. This chapter discusses the key takeaways of each chapter which led to these final conclusions.

Chapter 3 Synopsis: Effects of grade inclination on ACL loading

Aim 1 of this thesis surrounds the biomechanical response of the knee joint during varying ADLs. This study directly addressed this aim by successfully examining the relationships between kinematics, total knee loads, joint contact pressures, and ligament loads for level, inclined, and declined gait conditions. As expected, knee flexion angle and corresponding VGRF were key factors distinguishing joint biomechanics of each ADL. Pressure map data showed the medial compartment as the most impacted by changes in surface grade. For each ADL, ACL loads only increased in phases of gait corresponding to the lowest compression values. This meant that
although inclined gait produced higher VGRFs, total knee loads, and joint contact pressures throughout stance, ACL loads were virtually immeasurable at these time points.

This type of compressive shielding has also been demonstrated in previous studies of both cadaveric and sheep knee testing\textsuperscript{70}. It may be explained by compression bringing the origin and the insertion of the ligament closer together, thereby reducing the tensile load transmitted through the structure at these times. However, another explanation for low ACL loading during stance may be artefact caused by “expansion” of the remaining bones after removal of the articulation to achieve the ACL-isolated condition during in vitro simulations. Without the femoral condyles present to properly fill the joint space between femoral origin and tibial insertion points, the remaining uncompressed bone may have been free to expand further into the joint space, bringing the two ends of the ligament closer together and reducing overall tension on the ACL.

The largest limitation of this study was the low statistical power caused by high levels of variability among specimens. Still, this methodology has proven effective in demonstrating differences in functional ACL demand between varying ADLs. Results suggest that, while varying degrees of inclination produces significant kinematic differences throughout the gait cycle in the sagittal plane, differences in ACL loading are less observable due to compressive shielding during stages of ADLs when higher proportions of body weight are applied to the joint. Thus, inclined gait showed the greatest challenge to the ACL during hoof strike (0% gait), while declined gait showed the greatest risk to the ACL occurs during push-off (50% gait). This finding challenges the notion that ACL loading is strongly linked to ground reaction force, a common input to computational ACL models, which has implications for designing future ACL replacement techniques. According to these results, the greatest potential for activity-driven functional
deviation lies within the transition periods between unloaded and loaded knee states. This is consistent with many theories surrounding ACL injury prevention.

Chapter 4 Synopsis: Robotic Compliance and Bone Bending

Our laboratory utilizes robotics to reproduce measured in vivo kinematics, allowing further in vitro assessment of a variety of joint biomechanical properties in more physiologically relevant scenarios. However, there may be limitations in the types of activities able to be simulated, as high loading may induce kinematic error into the system. Even small deviations from in vivo motion could lead to erroneous conclusions of the native knee biomechanics. Therefore, this study aimed to measure the extent to which physiologic loading using a serial robot could produce deviation from the targeted kinematics. Results suggested that any deviations occurring are not attributable to excessive joint loading, confirming kinematic accuracy of in vitro testing for both Aims 1 and 2 of this thesis, and leaving open the possibility of simulating more athletic tasks without sacrificing accuracy.

Errors in the orthogonality of the mathematically reconstructed bony coordinate systems during gait simulation resulted in limited data available to truly assess the relationship between joint loading and altered kinematics from target values. Even with computational challenges, a subset of data was successfully presented which compares actual kinematic output with target values during simulations of anatomic motion. Results showed that kinematic deviations were not significantly linked with the amount of load experienced at the knee joint. In fact, most deviation occurred during flexed periods of gait when loads were low – namely mid stance and swing. These errors may be explained by the flexion of the specimen interfering with the line of sight between one or more markers and the more posteriorly positioned cameras.
Results suggest that the kinematics of simulated activities producing high loads at the joint are not at an increased risk to be influenced by bone bending and/or robotic compliance. Periods of high-loading did not correspond to decreased orthogonality, nor did they correspond to increases in kinematic deviation. However, future studies employing higher resolution equipment are necessary to confirm these findings.

Chapter 5 Synopsis: Comparisons between Sheep and Human Knees

This work used the sheep model as a surrogate to draw conclusions about biomechanics in human patients. To assess the validity of this inference, a study was performed to compare the primary and secondary restraints to motion in each DOF. Results support both Aims 1 and 2 of this thesis. Analysis of the stance phase of gait reveals more similarities between species than in the swing phase of gait. The combination of the bony interaction, menisci, and ACL accounted for almost all of the primary and secondary stabilization in the knee during stance in both species, supporting the sheep model as a surrogate for human knee joint biomechanics sustaining injury.

The large load contributions from the bony interactions during stance phase demonstrate how the compressed state of the knee can shield soft tissue structures from loading. This finding supports the hypothesis surrounding compressive shielding presented in the previous chapter. A key difference between the species was that the ovine MCL is more active than the human MCL while the ovine medial meniscus was less critical. This phenomenon could be explained by the sheep’s “knock-kneed” gait which naturally exposes the ovine knee to higher levels of abduction. Despite this, joint pressure occurs primarily in the medial compartment, which is also where most OA occurs in human patients. The major limitation of this study was the amount of load variability recorded within sheep and human samples, as specimen specific kinematics were not available.
Results of this study support use of the ovine (sheep) stifle (knee) joint as a biomechanical model for human knee dynamics, as the loaded structures in the knee during stance are similar between species. By using the sheep model as a surrogate for measuring in vivo and in vitro biomechanical function, researchers may be able to further develop the functional standards needed for designing repair procedures. This model may also serve to evaluate the safety and efficacy of future repair strategies.

Chapter 6 Synopsis: Effects of Population Variability in In Vitro Testing

As demonstrated within Chapters 4 and 5, applying a standardized motion to an entire sample of joint specimens can result in widely varying load dynamics within the study. This significantly lowers the statistical power of such in vitro test methods, making treatment differences difficult to identify without a very large sample size. This is particularly relevant for cadaveric testing, as no in vivo motions will exists for any individual specimen tested – leaving a population average motion as the only in vitro test option. Therefore, the goal of this study was to quantify the sources variability, so that future cadaveric studies may be able to account for the natural anatomical and/or biological differences present among knees of a population, potentially tailoring kinematic inputs to achieve physiologically relevant ADLs for each specimen. This is important for verification/validation of the results of Aim 1 in addition to future studies throughout biomechanics research. Tibio-femoral geometry and 6 DOF joint stiffness were examined as predictors of intact knee load variation during a simulation of human gait motion.

Results aligned with previous research correlating overall joint size and femoral notch height with recorded kinematics. However, joint stiffness metrics were much more powerful compared to anatomic geometry at predicting variations in joint dynamics during simulated in
vivo motion. Topography of the joint articulation appeared to be related to compression values during load bearing segments of activity. Ad/Abduction stiffness predicted medial/lateral joint dynamics with its associated torques. And translational laxity along with a joint size scale factor is loosely predictive of anterior/posterior dynamics with associated torques during gait.

Results of this study identify the unique patient attributes which impact the functional requirements of the knee and the importance of tailoring in vitro test methods to represent physiologic scenarios. Models established in this study may be further developed to tailor biomechanical test methods to individual specimens, thereby increasing our ability to model physiologic conditions and improving predictive capacity of in vitro screening for new and novel repair techniques.

**Chapter 7 Synopsis: Tracking Joint Remodeling using VGRFs**

Aim 2 surrounds the influence of the medial meniscus (MM) and medial collateral ligament (MCL) on the overall biomechanics of the knee joint, ACL forces and torques, and cartilage health. This study supports Aim 2 by examining the gradual changes in gait biomechanics due to injury to these surrounding structures and assuring that change is observable. VGRFs for Normal, MM-injured, MCL-injured, and Dual-injured animals were tracked over a 12-week healing period to quantify the amount of biomechanical changed induced by these injuries.

Results showed injury did not influence peak load or end of stance VGRFs, but did result in time-dependent differences at the beginning of stance. This is analogous to the time point with the animal begins to push itself forward, a critical time for knee function and biomechanics. For all activities, the MCL group demonstrated the least amount of change throughout recovery while animals without a MM (both MM and Dual groups) experienced significant change. No changes
were observed in the Normal control group, indicating that these observed differences were not due to training effects induced by improvements in animal cooperation.

This study supports the hypothesis that injury to surrounding structures induces changes to knee function during healing, which may ultimately impact demands on the ACL in a way that in vitro testing alone cannot evaluate. It also identifies 8 weeks as a general guide for allowing changes to occur, which will be an important consideration for future studies.

Chapter 8 Synopsis: Effects of MM and MCL Injury on ACL Loading

This study directly addresses Aim 2 by examining the relationships between VGRFs, kinematics, total knee loads, joint contact pressures, and ACL loads for Normal, MM-injured, MCL-injured, and Dual-injured animals. Results are then compared to regional development of OA in each injury group to identify cause and effect relationships among biomechanical metrics.

Results showed that, from a clinical perspective, patients sustaining MCL injuries may be more likely to see increased ACL loading during load bearing activities. However, no increase in OA progression is expected. Alternatively, patients sustaining isolated MM injury or meniscectomies are more likely to see increased ACL loading during transitions from distracted to compressed states. And although patients sustaining Dual injuries may not see any increase in ACL loading during, both MM and Dual patients may expect to develop similar levels of OA within the medial compartment of the knee.

As OA was primarily driven by the lack of a meniscus, ACL reconstructions have little opportunity to mitigate the early onset of OA, as the ACL does not contribute to loading during stance in groups missing menisci. However, to avoid subsequent graft failure, ACL
reconstructions for MCL-injured patients should be designed to sustain loads of up to 5% body weight, while MM-injured patients should sustain loads of up to 10% body weight.

This study contributes to a more thorough understanding of knee biomechanics and testing strategies as they relate to ACL function, and provides preliminary data for defining design requirements for future ACL reconstruction techniques.
Chapter 10

Perspectives and Recommendations

Robotic Simulations in the Sheep Model

The use of the sheep model in this work (Chapters 3, 7, and 8) has proven to be a major advantage when considering biologic effects of healing and applying physiologic motions directly measured from in vivo conditions. While clear differences are observable in the sheep knee compared to the human knee (including decreased laxity and increased flexion and abduction), these factors did not significantly influence overall joint biomechanics or the relationships between structure and function within the joint. In fact, differences only made this model more advantageous by accelerating healing response and progression of OA. Cartilage degeneration is impossible to observe in cadavers and occurs at a much slower rate in human patients. Thus, the sheep model as the clear choice in continued biomechanics research focused on eliminating or slowing the progression of early onset OA.

The next step in ACL reconstruction (ACLR) design is to actually perform ACLRs in the sheep model. While this work has examined changes in native ACL loading demands based on activity and injury level, it is also important to understand the biomechanical risks of not having an ACL at all. By utilizing the same methods presented in Chapter 8, we can quantify these risks by imposing ACL injuries and ACLRs and assessing the same metrics. This future work can be used in conjunction with the altered loading demands measured in this dissertation to establish which ACLR attributes are really important for avoiding OA. A follow up study may then be performed to assess ability of potential graft designs to restore native biomechanics.
While there is still much to be done in the sheep model, as knee biomechanics research progresses, it will be essential to move to cadaveric testing to translate findings and to evaluate ACL repair techniques for human patients.

**Robotic Simulations in the Cadaveric Model**

Successful translation of sheep model findings to the cadaveric model will be critical in establishing design criteria for human patients. Studies utilizing cadaveric specimens (Chapters 4, 5, and 6) successfully demonstrated the ability to perform robotic simulations to assess biomechanical properties in vitro. Additional challenges with using cadaveric specimen include the unavailability of individualized motion paths, increased variability due to pre-existing pathologies and patient histories, and increased risk of kinematic and/or force measurement error due to an increased distance from the joint center point and the end effector/force transducer. However, this work has determined these risks to be low when following the methods outlined.

The greatest challenge remaining in utilizing cadaveric specimen in robotics is the potential effects of using a population average motion to drive test kinematics. The sheep model will be a critical tool for assessing these effects. An important pre-requisite for applying position controlled robotic test methods is that applied motions are representative of in vivo function. Unlike with animal models, there is no feasible way to directly record in vivo kinematics for each cadaveric specimen. Therefore, it is critical to ensure that averaged population kinematics can be applied to a wide range of test specimens while continuing to reasonably represent physiologic conditions for the entire sample set. While Chapter 6 explains how specimen characteristics can drive kinetic variation, further studies are needed to better quantify the trade-offs associated with applying a population average vs. specimen specific (native) kinematic sets. Such a study could utilize the
sheep model to directly compare the kinetics resulting from and averaged and native test motions. This would allow researchers to positively identify variation stemming from kinematic mis-matching as opposed to natural kinetic variation inherent within the population. If mis-matched kinematics is a major cause of variation, a kinematic tailoring procedure may be useful to achieve relevant data surrounding physiologic biomechanics within future cadaveric studies.

By exposing human tissues to the mechanical context encountered during normal activities, researchers can better establish the patterns and limits of expected usage which govern functional tissue engineering parameters (FTEPs). These FTEPs are crucial to provide design criteria for the development of more effective strategies for the prevention and treatment of knee injuries.

**Sports Injury and Athletic Activities**

Results of Aim 1 indicated that the transition from unloaded to compressed knee state poses the greatest challenge to the ACL. This finding is consistent with many theories surrounding ACL injury mechanics in the world of sports medicine. While gait does cycle between unloaded and compressed conditions, the transition may not be drastic enough to pose a real challenge to the ACL. If clinicians are to keep patients out of their offices for repeat ACL tears, repairs must be robust enough to withstand more athletic ADLs. Because sheep are unable to perform such complex ADLs on demand, this work must be conducted in the cadaveric model which comes with additional challenges as presented in the previous section. While effects of population average kinematics have yet to be quantified, some work has already been done by Bates et. al. in this area.

A study successfully simulating drop vertical jumping and side step cutting in human cadaveric specimens showed that the ACL made significant contributions to anterior and medial
forces which is consistent with known ACL function and the work presented here. However, ACL loads only reached 3% body weight, approximately half the value of human ACL loading at heal strike of gait (Chapter 5). This smaller value is likely due to the kinematic alterations which were necessary to eliminate skin motion artifacts and successfully simulate these motions without destruction of the knee joints. These results highlight the importance of developing techniques to apply physiologically relevant kinematics, potentially tailored to each test specimen.

Focusing on more athletic tasks in cadaveric knees with help researchers and clinicians better understand how to transition safely between uncompressed and compressed knee states. This robotic testing platform should continue to be developed and utilized to examine many more factors governing ACL function and repair.

**Clinical Outcomes**

This work identifies biomechanical mechanisms which will build the framework for developing novel ACL reconstruction techniques in future generations of repair. Aim 1 focuses on the impacts of varying ADLs on ACL function and the translation of these findings to human knee while Aim 2 focuses on the impacts of surrounding knee structures on ACL function and regional OA risk. Both aims examined these factors using in vitro simulations of in vivo kinematics to represent physiologic conditions and measure loads in all DOFs—both critical pieces of information currently missing in the field of tissue engineering.

Results build on previously established characteristics of the ACL to further identify risks in patient activity and concomitant knee injury. These studies have shown that ACL loads
increase during transitions between weight bearing phases of activity and that compressive shielding of the ligament occurs during the sustained compression of stance phase. Because of this, stance-phase knee biomechanics and OA prognosis of patients undergoing meniscectomy or lacking MCL function may not be improvable by enhancements to ACL reconstruction techniques alone. However, these reconstructions can be expected to endure an increase in functional loading of between 5% and 10% body weight, which is an important consideration to avoid ACL graft rupture in patients sustaining concomitant injuries.

Future work may compliment these findings of native ACL loading patterns by further characterizing biomechanical risks promoting OA in ACL deficient patients. This two-pronged approach of 1) defining baseline needs and 2) mitigating potential risk will optimize design criteria for ACL repair, potentially preventing the early onset of OA in ACL-injured patients.


140. Nesbitt, R.J. "Impacts of stifle joint remodeling on vertical ground reaction forces following mcl transection and medial meniscectomy." in Orthopaedic Research Society 2013 Annual Meeting. 2013: San Antonio, TX.


