Mohammadsafa T Herfat, hereby submit this original work as part of the requirements for the degree of Doctor of Philosophy in Biomedical Engineering.

It is entitled:
Characterizing the Ovine Stifle Model as a Preclinical Biomechanical Surrogate for the Human Knee

Student's name: Mohammadsafa T Herfat

This work and its defense approved by:

Committee chair: Jason Shearn, PhD
Committee member: David Butler, PhD
Committee member: Donita Bylski-Austrow, PhD
Committee member: Marepalli Rao, PhD
Characterizing the Ovine Stifle Model as a Preclinical Biomechanical Surrogate for the Human Knee

A dissertation submitted to the
Division of Research and Advanced Studies
of the University of Cincinnati
in partial fulfillment of the
requirements for the degree of
DOCTOR OF PHILOSOPHY (Ph.D.)
in the Department of Biomedical Engineering
of the College of Engineering & Applied Science
2011

by
Safa T. Herfat
B.S., University of Cincinnati, 2005

Committee Chair: Jason T. Shearn, Ph.D.
Abstract

The long term goal of this research is to protect knee joint surfaces after knee surgery, thereby reducing the incidence of osteoarthritis. The objective of this dissertation was to determine if the ovine stifle joint is a suitable preclinical biomechanical model for the human knee. Using a 6 degree of freedom (DOF) robot, we applied simulated human and ovine in vivo motions to human knee and ovine stifle joints to measure the 3D joint and ACL kinetics. In addition, we investigated the biomechanical contributions of the other major knee structures. The in vivo studies were designed to determine the effect of surgically implanting motion and force sensors on ovine gait by monitoring the vertical ground reaction forces (VGRFs). Following surgery, we simultaneously measured VGRFs, knee kinematics, and the output from an arthroscopically implantable force probe (AIFP) which was implanted into the ACL. The kinematics were then simulated and applied to the operated joints, while measuring the 3D joint forces and moments. The AIFP output was used to validate the reproduced motions. Finally, we determined the effect of perturbing a simulated in vivo motion on 3D joint and ACL kinetics, which allowed us to investigate the potential effect of motion recording and reproduction errors on force and moment measurements.

Applying simulated human and ovine in vivo motions to human knee and ovine stifle joints resulted in few significant kinetic differences between the human and ovine intact joints and ACLs. For a simulated 6 DOF ovine motion applied to the ovine stifle joint, the bony interaction and medial meniscus were the major restraints during the stance phase of gait, whereas the MCL and ACL were the key restraints during the swing phase. The contributions of the ovine structures for a simulated 6 DOF in vivo motion are similar to the roles previously reported for the human. The in vivo studies revealed that surgery to implant motion and force sensors decreased average and peak VGRFs less than 10% and 20%, respectively, across all combinations of speed and grade. VGRF measurements acquired before and after surgery were consistent among animals, with a coefficient of variation averaging no more than
18% for all activities. Increasing treadmill speed only increased hind limb peak VGRFs, whereas increasing treadmill grade significantly increased hind limb average and peak VGRFs. Finally, we investigated the effect of motion recording and recreation errors on kinetic measurements acquired using a robot. The starting position of the in vivo ovine motion was adjusted (perturbed) in each degree of freedom to levels comparable to the extents of our motion recording and recreation errors. Perturbing a simulated in vivo motion in each degree of freedom indicated that only translational perturbations significantly affected the intact knee and ACL kinetics. Also, the average ACL resultant forces across all subjects and perturbations were less than 10% of the average ACL failure load.

This dissertation characterized the ovine stifle joint as a biomechanical surrogate for the human knee and validated a robotic methodology for measuring 3D joint and tissue kinetics using in vivo motions acquired during relevant activities of daily living (ADLs). Based on the 3D knee kinetics, the ovine stifle joint is a suitable biomechanical surrogate for the human knee. The novel methodology used to measure the 3D knee kinematics and kinetics for various ADLs has many applications to future orthopaedic research, notably in the areas of functional tissue engineering and sports medicine.
Acknowledgements

I would like to dedicate this dissertation to my parents who patiently persevered through great challenges during my upbringing. I would like to thank my family for making my educational achievements possible. My father instilled in me a dedication to diligence and a hunger for scientific achievement. My mother instilled in me the great sense of compassion that inspires me to pursue research in a field that provides the opportunity to improve the quality of life of others. Without their unwavering support and encouragement, my involvement in this research would not be possible.

I would also like to thank my research advisor, Dr. Jason Shearn, for his support and valuable mentorship throughout my graduate studies. Rather than micromanaging my every move, he allowed me the freedom to explore and quench my curiosities. I greatly appreciated his eagerness to teach as he was always available to answer questions. Not only is he very knowledgeable about biomechanics concepts and research, but he also enjoys teaching and providing valuable assistance in the lab; as he spends countless hours in the 3D Biomechanics Lab alongside his graduate students overcoming the many challenges which we encounter. I would also like to thank him for always being supportive as I pursued my ambitions, whether it was allowing me to teach High School math and science courses for a year as an NSF GK-12 Graduate Fellow or being supportive as I spent endless hours studying for the MCAT and completing medical school applications. As Dr. Shearn’s graduate student, I also had the opportunity to work with Engineers Without Borders. In the Spring of 2010, our service trip to Kenya was nearly cancelled because our faculty member had to withdraw at the last minute. Shortly after explaining the situation to Dr. Shearn, he agreed to serve as our faculty member and was a great asset to the team. While it has been a wonderful experience working for a talented professor and investigator, having a mentor whom shares a passion for service made it even more gratifying.

* This work was supported by grants from the National Institutes of Health (EB004859 and AR056660).
I would also like to thank Dr. David Butler for providing his mentorship and continually sharing his valuable knowledge in the field. Dr. Butler is an amazing educator which is why I have always recommended his courses to other students. In fact, completing his Tissue Biomechanics course during my undergraduate studies and learning about biomedical engineering research and its impact on the fields of orthopaedics and sports medicine inspired me to pursue my graduate studies.

I would also like to extend sincere thanks to Dr. Donita Bylski-Austrow for her continuous support and encouragement as a member of my PhD qualifier and PhD dissertation committees. Her support and recommendation throughout the medical school application process was also greatly appreciated. Thank you to Dr. M.B. Rao for also being a member of my PhD dissertation committee. I would also like to thank my fellow graduate students for their support and assistance in completing this research. I would like to thank Dan Boguszewski for the robotics training and his assistance in designing and conducting all of the robot experiments. Rebecca Nesbitt, also provided a valuable contribution to this research.

Special thanks to our collaborating surgeons, Dr. Marc Galloway and Dr. Michael Greiwe, for performing the surgeries to implant sensors, and providing their clinical perspectives to our in vivo studies. I would also like to thank Cindi Gooch and Denis Bailey for their valuable contributions to the in vivo studies. Lastly, I would like to thank everyone in the BME department for their support, notably Lori Beth Derenski, Linda Moeller, Kathryn Siefert, Shellie Smith, and Vernon Ferrell. Lastly I would like to thank Dr. Jing-Huei Lee for his support and mentorship during my first year of graduate school.
“Risk more than others think is safe.
Care more than others think is wise.
Dream more than others think is practical.
Expect more than others think is possible.”

Claude Bissell
# Table of Contents

Abstract .................................................................................................................................................. ii

Acknowledgements ................................................................................................................................. v

List of Tables ........................................................................................................................................ 3

List of Figures ...................................................................................................................................... 4

Chapter 1 – Background and Literature Review ....................................................................................... 6
  1.1 Prevalence and Effects of Knee Injuries .................................................................................. 6
  1.2 Knee Anatomy and Biomechanics ......................................................................................... 7
  1.3 Biomechanical Effects of Injury on Knee Function .............................................................. 19
  1.4 Clinical Metrics ...................................................................................................................... 19
  1.5 Current Surgical Treatments are Inadequate ......................................................................... 21
  1.6 Functional Tissue Engineering ............................................................................................ 24
  1.7 Preclinical Animal Models for Knee Biomechanics Research ........................................... 25
  1.8 Morphologies of the Ovine Stifle Joint and Human Knee are Similar .................................. 28
  1.9 Kinematics of the Ovine Stifle Joint and Human Knee are Similar ..................................... 30
  1.10 Research Objective ............................................................................................................. 33
  1.11 Specific Aims of Proposed Work ....................................................................................... 37

Chapter 2 – In Vivo Motions Produce Similar Intact Knee and ACL Kinetics between the Human and Ovine Model .............................................................................................................. 41
  2.1 Introduction .......................................................................................................................... 41
  2.2 Design & Methods ............................................................................................................... 43
  2.3 Results ................................................................................................................................. 48
  2.4 Discussion ........................................................................................................................... 51

Chapter 3 – Primary and Secondary Restraints in the Ovine Stifle Joint for a Simulated In Vivo Motion ................................................................................................................................. 55
  3.1 Introduction .......................................................................................................................... 55
  3.2 Design & Methods ............................................................................................................... 57
  3.3 Results .................................................................................................................................. 60
  3.4 Discussion ........................................................................................................................... 64
# Table of Contents

Chapter 4 – Effect of Surgery to Implant Motion and Force Sensors on Vertical Ground Reaction Forces in the Ovine Model ................................................................. 69

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1 Introduction</td>
<td>70</td>
</tr>
<tr>
<td>4.2 Design &amp; Methods</td>
<td>73</td>
</tr>
<tr>
<td>4.3 Results</td>
<td>78</td>
</tr>
<tr>
<td>4.4 Discussion</td>
<td>84</td>
</tr>
</tbody>
</table>

Chapter 5 – In Vivo Studies to Characterize the Biomechanics of the Ovine Model ........ 88

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.1 Measurement of In Vivo AIFP Output</td>
<td>88</td>
</tr>
<tr>
<td>5.2 Effect of AIFP Replacement on AIFP Output</td>
<td>90</td>
</tr>
<tr>
<td>5.3 Accuracy of Electromagnetic Tracking System for Knee Motion Measurement</td>
<td>91</td>
</tr>
<tr>
<td>5.4 In Vivo Knee Motion Measurement Using the Electromagnetic Tracking System</td>
<td>93</td>
</tr>
<tr>
<td>5.5 Applying Subject-Specific Simulated Motions to Measure Intact Knee and ACL Force</td>
<td>94</td>
</tr>
<tr>
<td>5.6 Proposed Solutions for Future Studies</td>
<td>96</td>
</tr>
<tr>
<td>5.7 In Summary of In Vivo Experiments</td>
<td>97</td>
</tr>
</tbody>
</table>

Chapter 6 – Effect of Perturbing a Simulated In Vivo Motion on Knee and ACL Kinetics ....... 99

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.1 Introduction</td>
<td>100</td>
</tr>
<tr>
<td>6.2 Design &amp; Methods</td>
<td>101</td>
</tr>
<tr>
<td>6.3 Results</td>
<td>104</td>
</tr>
<tr>
<td>6.4 Discussion</td>
<td>109</td>
</tr>
</tbody>
</table>

Chapter 7 – Discussion and Conclusions ............................................................................. 113

Chapter 8 – Perspectives and Recommendations .............................................................. 118

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.1 Identifying In Vitro Predictors of In Vivo Treatment Outcome</td>
<td>120</td>
</tr>
<tr>
<td>8.2 In Vivo Factors Influencing the Effectiveness of Orthopaedic Treatments</td>
<td>121</td>
</tr>
<tr>
<td>8.3 Characterizing the Normal Function of the Knee Joint and its Soft Tissue Structures</td>
<td>122</td>
</tr>
<tr>
<td>8.4 Determining the Biomechanical Effects of Knee Injuries</td>
<td>123</td>
</tr>
<tr>
<td>8.5 Evaluating Orthopaedic Treatments</td>
<td>124</td>
</tr>
<tr>
<td>8.6 Designing More Effective Orthopaedic Treatments</td>
<td>125</td>
</tr>
<tr>
<td>8.7 Translating Pre-Clinical Outcomes to Design More Effective Treatments</td>
<td>127</td>
</tr>
<tr>
<td>8.8 Summary of Future Directions</td>
<td>128</td>
</tr>
</tbody>
</table>

Bibliography .......................................................................................................................... 129
List of Tables

Table 1  Primary, secondary, and opposing structures during stance ..................................................... 61
Table 2  Primary, secondary, and opposing structures during swing....................................................... 62
Table 3  Effects of speed on response measures for a level surface (pre-surgery)........................................... 78
Table 4  Effects of inclination (mean ± sem) on response measures at 1.0 m/s (pre-surgery).................... 79
Table 5  Effects of surgery (mean ± sem) on response measures at 1.0 m/s (pre-surgery)....................... 82
Table 6  Effect of perturbations on intact knee kinetics ........................................................................ 105
Table 7  Effect of perturbations on ACL kinetics ................................................................................. 106
List of Figures

Figure 1  Major ligaments and menisci of the knee joint ................................................................. 8
Figure 2  Meniscus anatomy .......................................................................................................... 12
Figure 3  Human vs. ovine knee osteology ............................................................................... 29
Figure 4  Anatomical motions of the knee ................................................................................. 31
Figure 5  Distraction-compression force in the intact knee during stance and swing .............. 49
Figure 6  Anterior-posterior ACL force during stance and swing ............................................. 50
Figure 7  Medial-lateral ACL force during stance and swing .................................................... 50
Figure 8  Distraction-compression ACL force during stance and swing ................................. 50
Figure 9  Contributing structures to anterior/posterior forces ................................................. 62
Figure 10 Contributing structures to medial/lateral forces ....................................................... 63
Figure 11 Contributing structures to abduction/adduction moments ...................................... 63
Figure 12 Contributing structures to flexion/extension moments ........................................... 64
Figure 13 Fore and hind limb VGRFs ......................................................................................... 76
Figure 14 Sensor implantation ................................................................................................. 77
Figure 15 Effect of speed on VGRFs ......................................................................................... 79
Figure 16 Effect of grade on VGRFs ......................................................................................... 79
Figure 17 Inter-animal variability of VGRFs .......................................................................... 80
Figure 18 Effect of implantation surgery at 1.0 m/s on a level surface ....................................... 82
Figure 19 Effect of implantation surgery at 1.3 m/s on a level surface ....................................... 82
Figure 20 Effect of implantation surgery at 1.0 m/s on an inclined surface ............................... 83
Figure 21 Effect of implantation surgery at 1.3 m/s on an inclined surface ............................... 83
Figure 22 Normalized ACL AIFP output with the corresponding VGRF of the operated hind limb ............................ 89
Figure 23 AIFP implanted into a sagittal incision of a soft tissue structure ............................... 90
Figure 24 Reinsertion of an AIFP did not significantly change the AIFP output ..................... 91
Figure 25 Ovine knee kinematics ............................................................................................. 94
Figure 26 Intact knee and ACL force for a simulated in vivo motion ....................................... 95
Figure 27  C-D perturbations significantly affected C-D forces in the intact knee .......................... 107

Figure 28  A-P perturbations significantly affected A-P forces in the intact knee .......................... 107

Figure 29  A-P perturbations significantly affected A-P forces in the ACL................................. 108

Figure 30  ACL force-displacement curve...................................................................................... 108
Chapter 1

Background and Literature Review

1.1 Prevalence and Effects of Knee Injuries

The knee joint is one of the most commonly injured joints, especially during sports-related activities. Knee injuries account for an estimated 37% of all adult musculoskeletal soft tissue injuries [1]. The knee joint is the most common site for injury in adolescents and young adults, with knee injuries accounting for 15-30% of all sports related injuries [2]. Knee surgeries account for an estimated 60% of all sports-related surgeries in the general population [3]. Soft tissue injuries of the knee, predominantly injuries to the ligaments and menisci, account for the majority of knee injuries [2, 4]. Knee ligament injuries are frequently reported, constituting an estimated 45% of severe knee injuries [5] and are associated with a high level of short-term disability and need for surgical treatment and rehabilitation [2, 6, 7]. An estimated 250,000 cruciate ligament injuries are reported each year in the US, with the overall surgical and rehabilitative cost surpassing $4 billion annually [7, 8]. Meniscus tears are also very common with an estimated incidence of over 800,000 cases per year in the US [9].

These knee injuries can cause the early onset of osteoarthritis [10-12]. Osteoarthritis (i.e. degenerative joint disease or osteoarthrosis) is a clinical condition causing the degeneration of the articular cartilage within the joint. Following knee injury, the onset of osteoarthritis is most commonly attributed to mechanical wear due to altered knee biomechanics. Osteoarthritis is the most common form of progressive joint disease, affecting approximately 27 million people in the US [13]. Osteoarthritis of the knee can cause severe joint pain and inflammation, and eventually contribute to functional impairment and reduced independence [14]. Thus, osteoarthritis produces a lifelong, nonfatal disability [15-17] and thus has a very large socioeconomic burden.
Even after surgical treatment of knee injuries, there remains a very high incidence of osteoarthritis. For example, while ACL reconstructions have evolved with a reported success rate of over 90% [18, 19], these treatments fail to restore the normal biomechanics and address the long term implications of knee injury [20-26]. If surgeons do not restore the native biomechanics of the knee joint, the joint will remain susceptible to the onset and progression of osteoarthritis. Current treatment strategies, particularly ACL reconstructions, do not restore the native biomechanics of the knee joint [22, 27-29] and do not prevent the progression of osteoarthritis after injury [14, 30, 31]. The ultimate goal of this project is to improve long term outcome after ACL injury.

1.2 Knee Anatomy and Biomechanics

The knee is an anatomically and biomechanically complex modified hinge joint composed of two articulations occurring between 1) the distal femur and proximal tibia and 2) the distal femur and patella. The knee moves in 6 degrees of freedom during activities of daily living [32], yet provides stability and control as the joint experiences complex 3D forces and moments. There are several soft tissue structures that span across the tibiofemoral joint (Fig. 1), each of which contributes to normal knee function. Injuries to any of these structures can be detrimental to knee function and can increase the risk of injuring surrounding tissues [33-36], as well as cause the initiation and progression of long term complications such as osteoarthritis [20, 37]. The articular surfaces of the bones are lined with a thin layer of articular cartilage, which is prone to wear and osteoarthritis following injury [20].

While joint degeneration can occur at the tibiofemoral joint (joint composed of the tibia and femur, Fig. 1) or the patellofemoral joint (joint composed of the overlying patella and femur), this project will focus primarily on the tibiofemoral joint. The knee musculature and tendons are not studied specifically in this dissertation since our assumption is that the overlying muscles and tendons create the knee kinematics. Simulating and applying kinematics which were recorded in vivo with the muscles and tendons intact should compensate for their absence. The primary structure of interest for our
laboratory is the anterior cruciate ligament (ACL). However, due to the prevalence of injury to the other knee soft tissue structures, the selective cutting study (Chapter 3) will also investigate the biomechanical contributions of the posterior cruciate ligament (PCL), collateral ligaments, menisci, and joint capsule.

**Cruciate Ligaments**

Positioned centrally inside the joint capsule and within their own synovial membranes, the cruciate ligaments are vital to the stability of the knee joint. Each cruciate ligament is encased in its own synovial membrane [39], which provides a barrier between the ligament fibers and the synovial environment of the joint capsule. The ACL spans from the central anterior portion of the tibia to the posteromedial portion of the lateral femoral condyle. It is comprised of an anterior-medial (AM) bundle, which tightens as the knee flexes, and a posterior-lateral (PL) bundle, which tightens as the knee extends. The ACL functions as a major stabilizer for knee function, serving as the primary restraint to anterior tibial translation [40, 41] and a secondary restraint to internal rotation and varus-valgus angulation [40, 42, 43]. Coupled with the PCL, the ACL provides the “screw-home” mechanism which stabilizes the joint near full extension [44, 45]. The human ACL has also been reported to resist hyperextension during passive extension of the joint [46]. From an anterior view of the knee, the PCL crosses behind the ACL, extending from the lateral aspect of the medial femoral condyle to the posterior portion of the tibia just inferior to the tibial plateau. The PCL has a larger anterolateral bundle, which functions during flexion, and a smaller posteromedial bundle, which

![Figure 1](image-url)
functions primarily in extension [47]. The PCL primarily functions to prevent posterior tibial displacement [40, 41, 48, 49] but also serves as secondary restraint to external rotation and varus-valgus angulation [49, 50].

ACL injuries are one of the most common knee injuries and far more prevalent than PCL injuries [5]. Patients frequently tear their ACL and are prone to re-injury after surgery [51, 52]. PCL injuries account for only 15% to 20% of knee ligament injuries [53] and less than 2% of all documented knee injury cases [3]. Due to the much higher incidence of ACL injury, the majority of cruciate ligament research focuses on treating the ACL. The ACL generally heals very poorly [54, 55], lacking the ability to regenerate after complete ACL transection and exhibiting very slow and incomplete regeneration after partial rupture [55, 56]. The ACL’s poor healing ability has been attributed to several factors. First, the ACL has limited vascularization as its blood supply is restricted to small blood vessels on the ligament surface, with few vessels penetrating the midsubstance. This lack of blood supply can inhibit angiogenesis in partial ACL tears [55]. Also, an ACL tear can greatly compromise knee stability which can inhibit the natural healing process as the increased instability can increase the distance between the ends of the torn ACL. Many investigations have highlighted the differences between the ACL and the MCL, which exhibits much better healing capacity than the ACL. The location of the ACL within the synovial joint capsule can present different chemical factors than those encountered by the MCL in the extra-capsular environment [55]. Also, fibrocytes from the ACL and MCL differ in their growth rates, collagen expression, response to nitric oxide, and response to growth factors [55]. Therefore, the subpar healing ability of the ACL must be taken into account when designing treatments for ACL injuries.

Due to the limited healing capacity of the cruciate ligaments, surgeons frequently perform ligament reconstructions with the goal of restoring the normal biomechanics of the knee joint. More than 100,000 ACL reconstructions are performed annually in the US [57], whereas the limited number of PCL reconstructions performed annually has not been documented. About half of all ACL injuries
requiring surgery are caused by a noncontact injury mechanism [58, 59] which usually occurs with sudden deceleration, hyperextension, and excessive internal rotation of the tibia with respect to the femur [60]. Injury to the ACL is the most commonly reported sports ligament injury [3, 5, 58]. Common injury mechanisms for the PCL include a posteriorly directed impact to the proximal tibia of a flexed knee [61, 62] and the forced hyperextension or hyperflexion of the knee during contact sports [62, 63].

Due to the higher prevalence of ACL injury compared to PCL injury, studies investigating the biomechanical effects of cruciate ligament injury have focused primarily on ACL ruptures. There is a high incidence of knee osteoarthritis following ACL injury [10-12] because ACL injuries alter the kinematics of the knee joint [27, 28, 64-66]. Abnormal joint motion is observed immediately after full ACL tears [27, 64]. The altered kinematics and joint instability change the loading patterns of the knee, shifting the contact regions of the articular surfaces. This shift can cause elevated loading in regions of thinner articular cartilage, which are not conditioned to frequent load bearing and are thus susceptible to damage when loaded repeatedly or to higher levels [20, 67, 68]. Thus, this shift is commonly associated with mechanical wear in the joint which causes the early onset of osteoarthritis in the longer term [20, 30, 69, 70]. The altered loading environment can also increase the risk of injury to other soft tissue structures (e.g. a ligament or meniscus) [33-36, 71]. Concomitant injury to the MCL is very common, with combined ACL/MCL injuries accounting for over 95% of all multi-ligament injuries in the knee joint [72, 73]. The greater instability caused by these combined injuries causes more severe degenerative damage to the joint than isolated ligament injuries [74-76]. PCL injuries are usually concomitant with severe knee injuries of the ACL and MCL [60]. The isolated PCL injury is often more subtle and the resulting instability may be present during rapid direction changes, but drastic instability is more likely to be reported in cases of combined injuries [77].

Although cruciate ligament reconstructions have significant limitations in the long term such as degenerative joint disease, ACL reconstruction remains the most commonly applied treatment for
patients with ACL tears, while the use of PCL reconstruction to treat PCL tears remains controversial. There are many factors to consider when selecting the most effective treatment for cruciate ligament tears or ruptures. Approximately 80% of all knee ligament surgeries involve the ACL [58]. Without surgical intervention, ACL deficiency leads to wear of the articular cartilage, subchondral bone damage, and osteoarthritis [20, 69, 70]. Thus, an ACL reconstruction surgery using tissue autografts or allografts is recommended for most patients with ACL tears, with the goal of restoring normal ACL function. Although ACL repair strategies show early promise, with clinical studies reporting a 90% success rate [18, 19], failure to replicate the complex biomechanics of the normal ACL [20-22] has lead to long term failures and degeneration of the articular cartilage. Long term assessments have indicated that anterior-posterior laxity can increase after a couple years when using tendon autografts [31, 78]. In addition, current ACL reconstructions do not restore rotational stability [21, 22]. Therefore, current ACL reconstructions do not restore normal knee kinematics and frequently result in long term implications including osteoarthritis and re-injury. Even patients who undergo surgical reconstruction after sustaining an ACL tear have a 78% risk of radiographic osteoarthritis within 14 years following their injury [30]. Thus, conservative non-surgical treatment may be prescribed for older patients or patients who do not plan to continue sports activities.

In contrast to isolated ACL tears, an isolated partial PCL tear is predominantly treated without surgery [79]. Even though conservative (non-surgical) treatment is currently recommended for most PCL injuries, some investigators have suggested that the PCL-deficient knee is subjected to increased contact pressures [80, 81], and prone to developing progressive instability and osteoarthritis as a result of changes in knee kinematics that are not corrected by non-operative treatment. However, a PCL reconstruction is recommended in acute injuries that result in severe posterior tibial translation and joint instability. These objective criteria are often met in cases of combined ligamentous injuries, which constitute over half of all PCL injuries [82, 83]. Although athletes can often function at a high level with
an isolated PCL injury, combined injuries and isolated injuries with persistent instability usually require surgical treatment. Surgical treatment is also recommended for patients with persistent pain or discomfort that fails to improve with rehabilitation. As with any ligament reconstruction, the goal is to restore normal knee function, thus minimizing the long term deleterious effects of injury. However, the use of surgical reconstruction to treat an isolated PCL tear remains controversial because PCL reconstruction surgery does not substantially restore posterior stability in the long term [84-86]. Replacement of the PCL with the patellar tendon results in only a 23% success rate after 2 to 3 years [85]. Thus, there is little clinical evidence that suggests that PCL reconstruction significantly alleviates the progression of joint degeneration following PCL injury.

**Meniscus**

The menisci are fibrocartilaginous structures positioned between the femoral condyles and tibial plateaus (Fig. 2) to provide congruity between the articular surfaces of the bones. The medial and lateral menisci are semilunar-shaped structures with triangular cross-sections, increasing in height towards the periphery. This shape allows the menisci to increase the congruity between the round articular surfaces of the femoral condyles and the flatter tibial plateau throughout the full range of knee flexion [87]. The medial meniscus has several attachments which help to provide stability and limit its motion within the joint space: 1) continuous attachment of the periphery to the joint capsule, 2) anterior attachment to the anterior horn of the lateral meniscus and to the tibia, 3) medial attachment to deep fibers of the MCL, and 4) posterior attachment to the tibia between the medial and lateral tibial plateaus [88]. The lateral meniscus is smaller than the medial meniscus and lies on the
narrower lateral tibial plateau [88]. The lateral meniscus has a larger range of motion than the medial meniscus which has been attributed to its lack of attachment to the adjacent LCL and its loose peripheral attachment to the joint capsule [88, 89]. In addition, the variable lateral meniscus attachments to the medial femoral condyle provide anterior-posterior mobility [47, 87].

The complex fiber architecture of the menisci transmits complex biomechanics to stabilize the joint, distribute loads, and ultimately control joint kinematics. The menisci have non-parallel fiber architecture with disorganized fibers on the superior and inferior surfaces and fibers aligned circumferentially and radially in the midsubstance [90]. The wedge shape and the viscoelastic properties of the fibrocartilaginous meniscus tissue allow the meniscus to distribute loads between the femoral condyles and tibial plateaus. The meniscal attachments and the taller peripheries of the menisci help to stabilize the joint throughout the normal range of motion [88]. Each meniscus transmits various types of loads; simultaneously transmitting tensile, compressive, and shear forces through its complex fiber architecture [91]. By distributing and reducing the high contact stresses, the menisci help to protect the articular cartilage from wear [88].

The menisci serve a vital role in maintaining the normal function and health of the knee joint. While both menisci transmit and distribute the large compression forces in the joint, the medial meniscus also functions as a secondary stabilizer to resist anterior translation [92, 93]. In contrast, the lateral meniscus does not resist anterior translation of the tibia [92, 94], which has been attributed to its greater mobility compared to the medial meniscus. Medial meniscus deficiency causes varus instability in the knee [95], which can be attributed to the reduction in joint space in the medial compartment of a medial meniscus deficient knee. Resection of as little as 15% to 34% of the meniscus can increase the contact pressure transmitted through the tibiofemoral joint by more than 350% [88, 96].

Meniscus tears are very common, especially in the medial meniscus, but repair strategies have not proven effective in addressing the long term complications associated with meniscus injury. Over
800,000 meniscus tears are estimated to occur annually in the US [9], with tears frequently occurring during sports activities [3]. The medial meniscus is much more prone to tears than the lateral meniscus [3, 88]. The susceptibility of the medial meniscus is attributed to its limited mobility due to its multiple attachments to bone, the MCL and joint capsule. Thus, altered and elevated stresses caused by abnormal joint kinematics can cause the medial meniscus to tear. Meniscus injuries can be caused by contact or non-contact mechanisms [59], usually by an abnormal rotation of the knee while the knee is flexed. Concomitant injuries are common with over 75% of medial meniscus tears occurring with an ACL injury [97].

Several factors influence the healing capability of the meniscus following injury. The vascularization of the peripheral 20-30% of each meniscus provides a greater healing potential [98] compared to the inner portion. With adequate vascularization in the outer rim of the meniscus, surgeons can suture small tears in this region and allow the meniscus to heal. In contrast, the lack of vascularization in the inner portion of the meniscus compromises the healing capacity. Thus, a tear in this avascular region is usually arthroscopically excised by the surgeon [59]. However, surgeons commonly perform complete meniscectomies to treat large tears or severely damaged menisci in which torn portions of the meniscus are impinged in the joint, causing pain during activities of daily living. Although complete meniscectomy can alleviate joint pain in the short term, this treatment method reduces the joint space, increases contact loads, and alters knee kinematics. These implications increase the susceptibility of the knee joint to long term degradation [99-101]. Medial meniscus deficiency has been identified as a significant precursor to osteoarthritis and general debilitation of the knee [99-101], with 50% of patients developing osteoarthritis within 15 years [59]. Furthermore, meniscus repair and replacement strategies have not shown long term evidence of slowing the progression of OA [102-104]. Thus, meniscus tears represent a major clinical problem.
Collateral Ligaments

Lying outside of the joint capsule of the knee, the medial collateral ligament (MCL) provides stability to the medial aspect of the knee joint (Fig. 1). The MCL is composed of parallel superficial fibers and deep fibers, spanning from the medial femoral condyle to the medial proximal tibia. The deep fibers also attach to the periphery of the medial meniscus. The MCL serves as a major restraint to knee abduction and internal rotation [43, 105]. To a lesser degree, the MCL also resists anterior, posterior, and lateral translations, as well as external rotation [105-108].

MCL injuries are one of the most common knee injuries [5, 60] and much more prevalent than LCL injuries [5]. A major factor contributing to the much higher incidence of MCL injuries compared to LCL injuries is that MCL injuries are much more likely to occur during sports activities [5]. MCL injuries frequently occur as a result of both contact and noncontact injury mechanisms which can involve excess external rotation of the tibia, lateral knee impact and in extreme cases, knee dislocation [109]. Concomitant injuries to other structures in the knee are frequent and increase in likelihood with increasing severity of the MCL injury [109]. Combined MCL/ACL injuries are prevalent since the ACL serves as a secondary restraint to valgus rotation [110]. Furthermore, MCL deficiency increases the risk of ACL injury and ACL reconstruction failure due to the substantially increased loads that the ACL encounters in response to an anterior load or a valgus moment applied to the knee [36].

The lateral collateral ligament (LCL) also lies outside the joint capsule, providing stability to the lateral aspect of the knee (Fig. 1). The LCL is composed of parallel fibers extending from the lateral femoral condyle to the proximal, lateral fibula. The position of the LCL on the posterior-lateral aspect of the knee allows it to serve as primary restraint to knee adduction [43, 49, 105], a secondary restraint to external rotation of the tibia from full extension to 30° of knee flexion [43, 111], and a less important restraint to internal rotation [43]. The LCL also influences knee stability in the anterior and medial directions [105, 108].
Although LCL injuries are uncommon, these injuries can cause considerable joint instability. The LCL is one of the least commonly injured structures in the knee [1, 3], however, the resulting lateral joint instability causes greater joint instability than MCL insufficiency [62]. The LCL is predominantly injured by a varus rotation [60], but can also be caused by a knee dislocation. An LCL tear often occurs with injury to a cruciate ligament [60].

Isolated collateral ligament injuries are usually treated non-surgically because the collateral ligaments can heal naturally. Due to the much higher incidence of MCL injury and its superior healing capacity compared to the ACL, the healing response of the MCL has been studied extensively by investigators pursuing more effective treatments for ACL tears. The superior healing capacity of the MCL has been attributed to its abundant blood supply [55, 112], vascular and metabolic injury response [112] and extra-articular joint location [55, 113]. The MCL is well-vascularized for a ligament, with some vessels penetrating the ligament midsubstance. The ability of the MCL to increase its vascular volume and blood flow to the injury site through angiogenesis provides the ligament with a better healing ability than the ACL [112, 114]. The MCL is capable of healing naturally if the limb does not incur other injuries which compromise knee stability (e.g. an ACL tear) [109]. During an isolated MCL tear, it is speculated that the ends of a torn MCL remain in close proximity, rather than contracting and creating a large gap between ends like a torn ACL [55]. Although isolated collateral ligament injuries are predominantly treated non-surgically [109], controversy exists regarding the ideal treatment for concomitant damage to the anterior cruciate ligament (ACL) or posterior cruciate ligament (PCL). With the high prevalence of these combined injuries, treatment strategies for these combined injuries continue to evolve with research supporting both conservative and surgical repair/reconstruction of a collateral ligament [109].

**Joint Capsule**

The joint capsule of the knee is a thin layer of connective tissue enveloping the joint and helps to maintain joint health and contributes to joint stability [115]. The capsule is attached to the
peripheries of the distal femur and proximal tibia, enclosing the intra-articular environment of the knee joint. The medial and lateral menisci also attach to the capsule along their peripheries. The medial aspect of the joint capsule thickens and blends with the deep MCL fibers [47]. Anteriorly, the joint capsule is very thin and extends to form the suprapatellar pouch, which lies posterior to the patella and quadriceps femoris tendon. The inner synovial membrane of the joint capsule produces synovial fluid, which lubricates the articular surfaces of the joint and nourishes the articular cartilage [115]. The joint capsule also contributes to the overall stability of the knee joint [49, 115]. The posterior-medial region of the capsule provides stability as the knee is extended, restraining abduction, internal rotation and posterior drawer of the tibia [116].

Injuries to the joint capsule can be detrimental to the health of the knee joint. The capsule is often injured, leading to laxity, constriction and/or adhesion to surrounding structures [115]. Chronic wear or acute injury can cause a gap in the joint capsule, allowing the synovial fluid to escape the joint cavity, thus depriving the joint from the vital nutrients and lubrication the fluid provides. Joint capsule damage can lead to joint disease, including rheumatoid arthritis and osteoarthritis, crystal deposition disorders and bone spur formation [115].

**Osteology (Bone)**

The distal femur and proximal tibia provide the primary articulations for the knee joint but the contribution of bony contact to joint biomechanics has been minimally reported. The lateral tibial plateau is convex whereas the medial tibial condyle is concave [117]. The two tibial spines lie in between the medial and lateral plateaus, and surround the tibial insertion of the ACL. The femoral condyles are separated by the intercondylar notch on the distal aspect of the femur. Although investigators have frequently tracked the relative motion of the femur and tibia for kinematic analysis, the contributions of the tibiofemoral bony contact to joint stability and loading remain unknown.
The primary clinical condition associated with the knee osteology is osteoarthritis, which causes debilitating pain and limited mobility. The articular surfaces of the bone (i.e. articular cartilage) are prone to wear, especially for patients with a history of knee soft tissue injury. This progressive loss of articular cartilage will eventually expose the subchondral bone, causing joint pain as the subchondral bone remolds and osteophytes form on the damaged surfaces of the bone [118]. Advanced osteoarthritis can interfere with the ability to perform activities of daily living such as walking [119]. There are currently no consistently effective methods for slowing the progression of osteoarthritis and current treatments provide limited benefit for patients [120].

For patients with severe joint degeneration, the only treatment which can effectively relieve joint pain and restore mobility is to replace the articular surfaces and underlying bone with a knee replacement. Knee replacements can allow patients to return to less demanding activities such as walking. However, total knee replacements are primarily recommended for elderly patients due to the 10-15 year lifespan of most replacements [121] and the limited activity level recommended for patients with a knee replacement. If the patient has severe osteoarthritis in only a single compartment (medial or lateral) of the knee, a partial knee replacement can be performed. However, this type of knee replacement also has a limited lifespan.

The long term goal of this project is to design more effective treatments for soft tissue injuries of the knee, thereby preventing the early onset of osteoarthritis. Although the primary focus of this study is to investigate ACL biomechanics, injuries to other knee soft tissue structures can affect overall knee biomechanics and alter ACL loading. Thus, this project also focuses on determining the biomechanical contributions of the other soft tissue structures in the knee. More effective treatments are needed for these other structures, particularly the menisci.
1.3 Biomechanical Effects of Injury on Knee Function

**ACL Injury significantly alters knee kinematics during walking.** ACL deficiency alters the biomechanics of the tibiofemoral joint [28, 64, 122]. The ACL-deficient knee is significantly offset towards internal rotation relative to the healthy contralateral knee throughout the gait cycle for level walking [28]. The ACL-deficient knee also fails to reach full extension at midstance [28]. The ACL-deficient knee also exhibits greater varus, internal, and extension rotations than normal control knees during stance phase [27, 28]. These changes in joint kinematics also alter the joint kinetics. Using inverse dynamics strategies (using ground reaction force and joint kinematic measurements to calculate knee kinetics), investigators have computed that the ACL-deficient knee exhibits a significantly greater extension moment at heel-strike and a reduction of the flexion moment during midstance [123]. Since joint kinetics cannot be directly measured, other strategies are needed to further investigate the effect of ACL injury on joint kinetics.

1.4 Clinical Metrics

Clinical examinations of knee laxity are currently used to diagnose soft tissue injuries and to determine the effectiveness of repairs and treatments. Note that each clinical test subjectively assesses joint laxity by applying a load or torque in a direction which challenges the primary function of the structure of interest. To diagnose a soft tissue injury, physicians usually compare the laxities of the injured joint and the contralateral healthy knee. If the clinical exam indicates excess laxity of the injured joint, imaging modalities are then used to further investigate or confirm the presence of an injury. There are several clinical tests which are commonly used to diagnose soft tissue injury.

**Clinical tests are a valuable tool for the preliminary diagnosis of injury.** While magnetic resonance imaging (MRI) is a very accurate tool used to diagnose knee soft tissue injuries, clinicians will first use a clinical test to detect joint laxity prior to recommending a costly MRI. An MCL tear is
diagnosed by abducting the flexed knee while slightly externally rotating the foot. Alternatively, injury to the LCL is tested by adducting the knee in full extension and in 30 degrees of flexion with the foot in both a neutral position and in internal rotation. While there are several clinical tests than can detect an ACL injury, clinicians commonly apply the Lachman’s test [124]. The most popular variation of this clinical test is to stabilize the femur in slight external rotation while applying an anteriorly directed force to the proximal tibia with the knee positioned at 20-30° of flexion. A positive test is indicated by the lack of a definitive stopping point for the anterior displacement of the tibia or if the knee exhibits considerably greater anterior laxity (> 5 mm) than the healthy control knee [124]. The Lachman’s test is sensitive enough to also identify partial ACL tears. Similar to the Lachman’s test, surgeons can also perform the anterior drawer test by applying an anteriorly directed force to the tibia. However, the anterior drawer test is conducted with the knee positioned at 90° of flexion and is not as sensitive for the detection of an ACL rupture [60]. A PCL injury is detected using the posterior drawer test, which applies a posteriorly directed force to the tibia with the knee at 70-90° of flexion [61]. The quadriceps active test can also detect a PCL injury [60]. By positioning the knee at 60° of flexion, the clinician holds the foot flat against the table with the patient lying supine. As the patient extends the injured knee isometrically, a PCL deficiency is determined by an excess tibial translation causing the medial tibial plateau to suddenly lose contact with the medial femoral condyle [61]. A meniscus injury is usually assessed using the McMurray test or Apley test, both of which internally and externally rotate the knee at selected flexion angles. As the knee is slightly internally and externally rotated, pain or a popping sound serves as the primary determinants of meniscal injury. Chronic conditions such as osteoarthritis and even the calcification of a soft tissue structure (which indicates abnormal loading or healing) can also be diagnosed radiographically (i.e. X-ray) or arthroscopically [60]. While the clinical tests can be successfully used to diagnose a knee injury, they are also used as subjective tools to assess the effectiveness of treatments.
Clinical tests are inadequate for determining the effectiveness of treatments. Neither clinical tests, nor imaging modalities provide a way to assess if a repair or treatment restores the normal biomechanics of the knee joint in all 6 degrees of freedom (DOF). The clinical tests can roughly gauge if the stability of the knee has been restored. At best, radiographs and MRIs can detect healing or joint degeneration. Without an objective method to verify the restoration of the normal 6 DOF kinematics and associated loading environment of the knee and its individual structures, biomedical engineers and orthopaedic surgeons cannot determine the effectiveness of current repair strategies. The inability to restore the normal biomechanics of the joint can have detrimental effects on the health of the knee joint. Thus, patients continue to suffer from an early onset of osteoarthritis even after surgical treatment.

1.5 Current Surgical Treatments are Inadequate

ACL reconstructions do not restore normal knee kinematics. Using a bi-plane radiography system, Tashman et al. [125] determined that anterior tibial translation did not differ between reconstructed and healthy contralateral knees during downhill running. However, reconstructed knees were significantly more externally rotated and adducted than the contralateral, uninjured joints [126]. Also, anterior tibial translation increased 12 months after surgery [126]. While these studies provide results for an actual activity of daily living (ADL), downhill running is very stressful on the ACL [125, 126]. Unfortunately, the bi-plane radiography system has not yet been used to determine the effects of surgical repairs or reconstructions on knee kinematics for more relevant ADLs for the general population, such as level walking.

Gao et al. [28] investigated the differences in human knee kinematics between healthy control, ACL-deficient (ACL-D), and ACL-reconstructed (ACL-R) knees during level walking using reflective skin markers; a motion recording method which is less accurate than bi-plane radiography. The ACL-R knees
showed a significant flexion offset (i.e. timing of flexion varied) during swing phase compared to the healthy knees. In contrast to results using the bi-plane radiography system [125], ACL reconstruction did not correct the reduced external rotation exhibited by the ACL-D knees prior to toe-off. Although these changes may seem minimal, they are sufficient to shift bony contact regions which alters the biomechanics of the joint and its structures, and can cause osteoarthritis in the long term [20, 37].

**Current grafts used for ACL reconstruction have important limitations.** Although surgeons frequently perform ACL reconstructions in patients with ACL tears, current ACL reconstructions have limitations. Several graft options and surgical strategies are widely used to reconstruct the ACL. Current repair strategies may reduce some of the excess joint laxity caused by injury but do not adequately address the long term complications following knee injury, such as osteoarthritis. In addition to the long term complications, each type of graft used to reconstruct the ACL also has short term limitations.

The major limitation of all ACL reconstructions is that the procedure requires replacing the ACL, a biomechanically complex soft tissue structure that experiences 3D forces, with a tendon, which is a soft tissue structure that primarily experiences uniaxial tensile forces. Controversy exists regarding the most effective graft (autograft versus allograft) used for ACL reconstruction. Tendon autografts are currently the most widely used grafts for ACL reconstruction. The major complaint following reconstruction with an autograft is donor site morbidity, primarily when using the bone-patellar tendon-bone (BPTB) graft [23, 24, 26, 127]. Thus, the use of tendon allografts in ACL reconstruction has steadily increased over the last two decades [128-131]. However, allografts have other limitations which include the potential for disease transmission, prolonged graft healing, as well as lower knee function scores and higher graft failure rates than autografts. Allografts are harvested from cadavers, many of whom are older than the patient and whose tissues may exhibit considerably lower stiffness and strength. Compared to autografts, allografts cause significantly lower overall knee stability scores, which are determined from laxity tests performed by orthopaedic surgeons [128, 129, 132]. Although early
recovery time favors allografts, longer term recovery time favors autografts. There is evidence that allografts take longer to incorporate, revascularize, and remodel than autograft tissue [130, 132-136]. This longer period of incorporation and remodeling may cause allografts to have weaker structural properties than autografts and potentially lead to higher failure rates [131, 137, 138]. While the risk of infection when using an allograft material in ACL reconstruction is now considered minimal [139, 140], reconstruction using a BPTB allograft is associated with a higher graft rupture [129] and revision rate [128] when compared to using a BPTB autograft. Some issues also remain regarding the cost of sterilization and the effects of sterilization on the structural properties of the allograft tissue, which may contribute to the higher failure and revision rates [128, 141-143]. Thus, a better graft is needed for ACL reconstruction. The overall clinical goal of this project is to improve outcomes after ACL injury by establishing parameters which will be used to design more effective grafts.

The ideal graft for ACL reconstruction should have several characteristics. To minimize the long term degeneration following injury and repair, the graft must restore native joint biomechanics. The ideal graft should also be of sufficient size, incorporate fully and quickly within bone tunnels, and have no risk of immune rejection or disease transmission [132]. The graft should also minimize donor-site morbidity. Lastly, the graft should be relatively inexpensive and readily available. Unfortunately, a graft which meets these criteria does not currently exist.

Since normal joint biomechanics are not achieved by using tendon grafts, one alternative would be to design a graft which reproduces the function of the native ligament. Tissue engineering provides a technique whereby engineers use implanted cells, scaffolds, DNA and/or protein to replace or repair injured or diseased tissues and organs [144]. Another factor complicating the development of an ACL graft is that normal knee kinematics between subjects is highly variable [32]. Thus, the optimal graft may have to be custom-tailored to meet the specific demands of each patient. However, investigators must first determine the design criteria needed to develop more effective ACL reconstructions.
Functional tissue engineering may offer a solution to designing more effective grafts which can adequately restore the normal knee function of each patient. The aforementioned criteria would serve as design requirements for the development of the tissue engineered graft.

1.6 Functional Tissue Engineering

Functional tissue engineering offers an approach for treating orthopaedic injuries by using tissue engineered repairs or replacements to restore normal joint function [91, 144]. The ultimate clinical goal is to minimize the progression of joint degeneration (i.e. osteoarthritis) following injury. In order to design more effective treatments, we must first fully characterize the normal function of the joint and its structures. One approach to understanding the normal *in vivo* function of tissues is to conduct *in vitro* biomechanical experiments that mimic *in vivo* conditions. Investigators can use this method to establish functional tissue engineering parameters (FTEPs), which will serve as design parameters for treatment and rehabilitation strategies [91, 144]. The biomechanical goal is to restore the normal joint kinematics for actual activities of daily living (ADLs) and provide a safety factor for more vigorous activities, rather than to restore native tissue failure properties [144]. Critical among these FTEPs are the *in vivo* tissue forces and displacements for a variety of ADLs. Unfortunately, *in vivo* forces cannot be directly and accurately measured in humans because it requires harvesting the joint to calibrate the force sensor. To overcome this obstacle, preclinical animal models can be used to measure *in vivo* tissue forces. Once these parameters have been established for normal tissues, investigators can then use them to develop treatment strategies which can meet the requirements of the normal *in vivo* condition.

The FTEPs can be used to establish both design parameters and evaluation benchmarks, as well as to manufacture tissue engineered grafts. For a graft to restore the native biomechanics of the joint, it must be designed to meet criteria established based on *in vivo* conditions. The actual *in vivo* loading environment can be reproduced and applied to stimulate tissue engineered therapies as they develop in culture. The *in vivo* loads can also be applied to tissue engineered constructs to screen their
effectiveness prior to implantation. After meeting the \textit{in vitro} screening requirements, the constructs can be implanted in preclinical animal models to evaluate their effectiveness in restoring the normal \textit{in vivo} kinematics of the knee. Similar strategies will then be used when the repair strategy is evaluated in patients during clinical trials. Thus, FTEPs will be vital in the design, production, and evaluation of more effective orthopaedic treatments [91].

1.7 Preclinical Animal Models for Knee Biomechanics Research

In order to establish FTEPs that can be used to evaluate and develop treatments for the human knee, investigators require preclinical animal models which replicate functional aspects of the human knee. Researchers have characterized animal models and compared aspects of knee anatomy, biomechanics and biology across species [145-147]. However, there is no gold standard surrogate for the human knee.

Each large animal model used for knee biomechanics research has advantages and disadvantages. A suitable biomechanical surrogate for the human knee should have an appropriate stifle (knee) joint size allowing for surgical treatments and the biomechanical analysis of the joint and its soft tissue structures. Primates and horses meet many of these requirements but are far too expensive for large scale studies. The remaining large, readily available animals are the dog, pig, goat, and sheep. The canine (dog) model is easily trainable, intra-articular knee surgery is convenient and clinical outcomes are well published. However, there is a likelihood of emotional attachment and using this animal is ethically controversial [148]. The porcine (pig) model is widely used due to the low cost and age-, weight-, and gender-matched animals are readily available [149]. The porcine stifle model is similar in size to the human knee and contains the same major soft tissue structures as the human knee [147, 150]. The porcine model also has hematological characteristics similar to the human [151], which is particularly important for healing studies. Also, forces in the porcine knee joint are biomechanically
similar to the human knee when combined axial compressive and anterior tibial loads are applied [152].
The porcine stifle is also an ACL-dependent joint [153]. However, few in vivo biomechanics studies have
used the porcine model as pigs are difficult to handle and the large, readily available pig breeds have a
rapid growth rate [149]. Furthermore, ACL reconstruction studies must account for the fact that the
strength of porcine tendons is half that of human tendons [154]. An advantage of the caprine (goat)
model is that the cartilage thickness is closer to human than other animal models [148]. However, goats
are not as readily available and more difficult to handle than their ovine (sheep) counterparts [148].

The ovine model was selected for this project for numerous reasons. Advantages of using sheep
include availability, ease of handling and training, the vast amount of published clinical outcomes, and
the ease of performing intra-articular surgery in the ovine stifle joint [145, 155, 156]. The ovine stifle
joint also contains the same major soft tissue structures as the human knee [155]. Of most relevance to
this project, the in vivo stifle joint kinematics and ground reaction forces have been measured [146,
157]. Having performed in vivo studies using both the sheep and goat model [158-160], we have
learned that sheep are more manageable and easier to train during controlled gait experiments. Also,
sheep are now much more readily available and less costly than are goats.

Suitability of the Animal Model for Future Biomechanics Research

The future directions of this project will require an animal model which can be used to
determine the effects of injury and to evaluate treatment strategies. Investigators have used multiple
animal models to investigate the degenerative effects of injury and the biologics of healing. Like
humans, quadrupedal animal models have also been shown to experience altered tibiofemoral
kinematics due to ACL deficiency. ACL transection altered the tibiofemoral kinematics of the canine
[122, 161] and changed both the tibiofemoral kinematics and kinetics in the feline [162]. ACL
transection in animal models also resulted in wear of the articular cartilage leading to osteoarthritis
[163, 164] and caused biological changes associated with the onset of osteoarthritis [163, 165]. Using
the ovine model, early onset of OA has also been induced using various techniques, including radial medial meniscus transection [166], medial femoral condyle impact injury created by arthrotomy [167], medial meniscectomy [168-170] and lateral meniscectomy [171].

While investigations using animal models have provided valuable knowledge about the effects of injury, many gaps in knowledge remain about the normal biomechanics of the knee and its structures, the effects of injury and treatments, and the etiology of osteoarthritis. In pursuit of characterizing the normal biomechanics of the knee and its structures, this project seeks to investigate the ovine stifle joint as a possible biomechanical surrogate for the human knee. In future studies, we will also investigate the biomechanical effects and biological aspects of injury and healing. However, as with any animal model, there are limitations to consider when interpreting the results of studies using preclinical animal models. Findings in a quadrupedal model should not be directly correlated to the human knee.

Several studies have reported notable findings using the ovine model which will be relevant to our future orthopaedic investigations. The ovine stifle joint is an ideal experimental model for studying a range of orthopedic conditions and treatments [145]. Both arthrotomy incision and arthroscopy techniques provide excellent access to the major structures within the joint [145]. Reconstructions or replacements of the cruciate ligaments [156, 172-174], MCL [173] and menisci [175] have been performed in sheep. In addition, the effect of medial meniscectomy on joint contact load distribution has also been assessed in the ovine joint [176]. Also, the main structural features that will influence meniscus repair (e.g. cellularity, vascularity, collagen structure) are similar in sheep and human [177].

The ovine stifle joint is also a suitable model for investigating the progression [178-180] and treatments [181, 182] of osteoarthritis. Osteoarthritis can be easily induced in the ovine articular cartilage [166, 167, 169-171, 179]. The ovine model can also be used to investigate the pathogenesis and treatment of osteoarthritis [181, 182], as well as treatments for chondral and osteochondral lesions [183, 184]. Investigators have also used the ovine model to investigate bone healing [185-187] which
has applications to the incorporation of soft tissue grafts with bone plugs (i.e. BPTB grafts). These are reasons that have influenced our choice of the ovine model.

1.8 The Morphologies of the Ovine Stifle Joint and Human Knee are Similar

The ovine stifle joint has been increasingly used as a large animal model for orthopaedic research [188]. The ovine stifle joint structure shares many similarities with the human knee, including its overall anatomy [155]. Both joints are comprised of patellofemoral and tibiofemoral joints with a common joint capsule. An investigation of the ligaments, joint stability and ACL strength of the ovine stifle joint revealed that the ovine stifle is a valid model for human cruciate ligament surgery [155]. As in the human, the cruciate ligaments are enclosed within their own synovial sheaths inside the joint space. The extension of the ovine stifle is also limited by the collateral and cruciate ligaments, and ACL transection results in increased extension of the joint [46, 145, 155]. The ovine ACL also consists of two distinct fiber bundles with attachments and orientations similar to the anteromedial (AM) and posterolateral (PL) bundles of the human ACL [155]. The two ovine ACL bundles exhibit a similar reciprocal action as the anterior bundle tightens with joint flexion while the posterior fiber tightens with joint extension [155, 189]. The ovine ACL also has a similar ultimate strength as the ACL of young adult humans (ovine: 2.4 kN [155]; human: 2.5 kN [190]). The collateral ligaments of the ovine stifle have attachments and orientations similar to those of the human knee. The morphology and attachments of the menisci are also similar between species [145, 155]. The menisci of both species lie between the articular surfaces to improve the congruency between the incongruent distal femur and proximal tibia [145], increase the surface contact area which distributes the load, and resist and absorb compression loads in the joints [88, 99, 191].

Although there are obvious differences between the size and morphology of the ovine and human tibia and femur (e.g. the tripartition of the distal femur and the narrow trochlea), the overall
osseous morphology of the ovine stifle and human knee joints share important features ([192]; Fig. 3). Most importantly, both joints have medial and lateral femoral condyles which articulate on the medial and lateral tibial plateaus, respectively. The femurs of both species also have distinct trochlear grooves allowing for similar articulations between the patella and femur [192]. The cortical bone thickness of the distal femur is not significantly different between species [192]. Also, the medial tibial plateaus of both joints have a larger contact area and experience larger compressive loads than the lateral plateaus [192]. These are important factors which can produce similar joint function and loading between species.

There are also important differences in the bones which can influence the joint biomechanics of each species. In the ovine joint, the articular cartilage of the medial tibial plateau is approximately two times thicker than that of the lateral plateau [192]. In contrast, the articular cartilage is thicker in the lateral plateau in the human knee [192]. The ovine stifle joint also has a massive bone stock below the tibial plateau and a proximal tibial shaft with remarkably thick cortical bone [192]. These differences in articular cartilage and bone thicknesses can cause differences in loading between species. These differences provide potential evidence that each species may exhibit different loading patterns, which could also influence their response to injuries and treatments.

It is important to also consider other differences between species. Humans are bipeds walking on flat feet and sheep are quadrupeds walking on narrow hooves and lack the ability to attain a straight-leg posture (43° at full extension, [146]). Thus, the load-bearing regions throughout gait differ between the ovine and human. The ovine stifle is also stiffer (higher forces for same motion path) than the

![Figure 3 Human vs. ovine knee osteology. A: human femur; B: ovine femur; C: human tibia; D: ovine tibia [192]](image-url)
human knee which will influence the kinematics and kinetics of the joint. Also, the fibula is a rudimentary structure in the ovine joint [192]. Since the LCL attaches to the lax fibular head of the ovine joint, the LCL may contribute less to joint stability than the human LCL. These factors indicate that the human knee and ovine model may exhibit significant differences in joint biomechanics. ACL transection also produces greater anterior-posterior laxity in the ovine stifle joint than in the human knee [155]. The additional anterior tibial translation caused by transection of the ovine ACL is approximately double the amount caused by transection of the human ACL [41, 155]. Thus, transection of the ovine ACL results in a grossly unstable joint. This occurs despite the secondary restraints providing a similar contribution to resisting tibial anterior displacement as for human knees [155, 193]. The combination of greater joint stiffness of the normal ovine and the proportionally greater instability caused by ACL deficiency in the ovine joint suggests that the ACL reconstruction may have to endure a more demanding environment to restore normal function in the ovine stifle joint compared to the human knee joint. However, *in vivo* loads must first be determined for activities of daily living to determine the normal knee function for each species.

1.9 The Ovine Stifle Joint and Human Knee Kinematics are Similar

Designing more effective treatments for knee injuries requires design parameters based on normal knee function during relevant activities of daily living (ADLs). Full 3D kinematics have been measured in patients during walking [32]. Subsets of these kinematics have also been measured during multiple ADLs [64, 194]. However, we lack full 3D kinematics for multiple relevant ADLs, and we cannot directly measure corresponding joint and tissue forces in humans. Many studies examine knee biomechanics during strenuous squatting, running, or stair walking activities [125, 195-197] even though these are activities that most individuals can forego and still sustain an excellent quality of life. Thus, it is important for investigators to focus on establishing design requirements for relevant ADLs. While
investigators continue to study the factors contributing to the initiation and progression of osteoarthritis, researchers speculate that the long term prevalence of OA after knee injury is caused by altered joint kinematics which lead to chronic mechanical and biologic changes of the joint [64]. The factors contributing to the gradual chronic degeneration of the articular surfaces of the knee joint should thus be analyzed for ADLs which are encountered repeatedly throughout everyday life. Based on this criterion, walking may be the most relevant ADL for studying joint degeneration.

The human knee and the ovine model both move in 6 degrees of freedom (DOF) during ADLs and exhibit substantial kinematic variability between subjects [32, 146]. The kinematics of the knee can be described according to the anatomical axes using a knee joint coordinate system developed in our lab (Fig. 4) [198]. The translations occur along the anterior-posterior (shown as the Y-axis), medial-lateral (shown as the X-axis), and compression-distraction (shown as the Z-axis) axes while the abduction-abduction, flexion-extension, and internal-external rotations occur about these axes, respectively. The 6 DOF joint kinematics of healthy normal knees have been accurately measured using reflective marker systems rigidly attached to bone pins [32]. However, due to the invasiveness of the methods, knee motions were only recorded for a single ADL (walking) and limited subjects were available. Furthermore, even though all subjects were young, male with bilateral healthy knees, there was considerable inter-subject variability across all 6 DOF. This indicates that even with a controlled

![Figure 4 Anatomical motions of the knee. Translations and rotations according to the joint coordinate system for the tibia and femur [198]](image_url)
group, gait patterns will vary between subjects. Thus, investigators seeking to measure joint forces and moments by reproducing in vivo conditions should ideally apply subject-specific kinematics to each joint. This methodology requires harvesting the joint to apply the in vivo joint kinematics. Since this cannot be performed in human patients, a preclinical animal model is required. The 6 DOF ovine stifle joint kinematics have been previously measured [146] with the same methodology used by Lafortune et al. [32]. The ovine stifle kinematics were reported with respect to the same joint coordinate system used to describe anatomical motions for the human knee [198].

Although the ovine stifle joint does not achieve a straight-leg orientation as seen in the human knee, the knee kinematics of the bipedal human and the quadrupedal sheep are similar during walking [32, 146]. In contrast to the human knee joint which achieves a straight-leg orientation at heel-strike, the ovine stifle joint is flexed near 43° at heel-strike [152]. However, similar to the human knee, flexion of the ovine joint is the largest motion (34.0°) during walking, followed by internal-external rotation (11.8°), and abduction-adduction (4.1°) [26, 152]. Although the range of flexion is approximately half that of the human joint during walking (34° vs. 60°), the flexion-extension pattern is similar throughout each gait cycle. During stance, both joints are extended at heel-strike (43° for ovine vs. full extension [≈ 0°] for human) and subsequently flex as the limb is loaded during stance, followed by extension again prior to toe-off [32, 146]. During swing, both joints flex to peak flexion and subsequently extend to the next heel-strike [32, 146]. Joint abduction and medial tibial translation is coupled with joint flexion in both joints [32, 146]. Hence, the kinematics are similar between the ovine stifle and human knee joint during a level walking activity. However, the full 3D joint kinetics of the ovine stifle and human knee have not yet been characterized for any ADL to determine if the ovine stifle joint is a suitable biomechanical model for the human knee.
1.10 Research Objective

This dissertation describes experiments designed to characterize whether the ovine stifle joint is an appropriate biomechanical surrogate for the human knee. This project tests aspects of the global hypothesis that similar morphology and kinematics between the ovine stifle and human knee joints will produce functional similarities for relevant activities of daily living. Many studies have measured *in vivo* knee kinematics in humans during an activity of daily living [28, 32, 64, 125]. For animal models, full 6 degree of freedom (DOF) kinematics have only been measured in sheep [146]. However, *in vivo* joint kinetics cannot be directly measured in either the human or sheep. Advances in robotics and load cell technologies now provide the capability to apply simulated *in vivo* motions to the joint to measure the 3D joint forces and moments. This dissertation will investigate the 3D joint kinetics of the ovine stifle joint and the human knee to determine if the ovine joint is a suitable biomechanical surrogate model for the human knee joint. The functional similarities between the ovine stifle and human knee will be determined by comparing the 3D joint forces and moments for the intact joints and their individual structures (e.g. ACL).

The objective of the first study (Chapter 2) was to use a novel *in vitro* robotics methodology to compare the biomechanics of the intact joint and ACL between the ovine stifle and human knee joints using simulated activities of daily living (ADLs). Investigators are unable to directly measure the 3D joint or ACL kinetics in patients and cannot isolate the knee or its structures to impose realistic physiologic loading patterns. Animal models offer an alternative to acquire similar measurements. In an effort to measure 3D forces and moments which are more representative of *in vivo* knee kinetics, we implement a methodology which allows us to apply 6 DOF *in vivo* knee motions to actual knee joints to measure the corresponding forces and moments of the intact joint and ACL. First, we applied a simulated *in vivo* ovine motion [146] to both the ovine and human joints to measure the 3D forces and moments for each joint and ACL. We also applied a 6 DOF *in vivo* human motion [32] to human knees to measure the 3D
forces and moments of the human knee joint and ACL. Our preliminary studies indicated that the ovine stifle joint was stiffer than the human knee because the same ovine motion produced considerably higher forces (approximately double the resultant force) and moments in the ovine joint compared to the human joint. Our underlying hypothesis that the knee joints of the human and ovine function similarly, led us to formulate the following hypotheses:

**Hypothesis 1**: Applying an *in vivo* ovine motion to both the ovine stifle joint and human knee will produce insignificant differences in joint force and moment patterns between species due to the similar anatomy of the ovine and human joints.

**Hypothesis 2**: Applying an *in vivo* ovine motion to both the ovine stifle joint and human knee will produce higher force and moment magnitudes in the ovine joint than in the human joint due to the ovine joint being stiffer.

**Hypothesis 3**: Applying both the simulated ovine motion and simulated human motion to the human knee will produce insignificant differences in joint force and moment curve shapes due to the similar *in vivo* joint kinematics between species.

**Hypothesis 4**: Applying the simulated ovine motion and simulated human motion to the human knee will produce larger force and moment ranges for the human motion than the ovine motion due to the larger range of motion.

The objective of the next study (Chapter 3) was to measure the force and moment contributions of the various soft tissue structures and bony contact of the intact ovine stifle joint. Each soft tissue structure in the knee performs a vital function during gait. To determine the effects of injury, as well as prevention strategies and treatments, we must first understand the contribution of each structure in the normal joint. However, our current understanding of the function of these structures is usually limited to measurements recorded during simulations of clinical tests rather than actual ADLs [42, 199-201]. Furthermore, previous studies predominantly investigated the contributions of only soft tissue
structures to knee biomechanics but few have investigated the role of bony contact [35, 40, 41, 43, 44, 49, 106, 108, 199, 202]. With the human and ovine knee both exhibiting significant compression forces during stance, the bony contact in both species should contribute significantly to the 3D knee biomechanics. In this study, we applied a simulated in vivo motion to the ovine stifle joint to examine the contributions of knee soft tissue structures and bony contact. We performed a selective cutting sequence using a stiffness-based, displacement-controlled methodology to determine the primary and secondary restraints to each of the 6 DOF of motion. The application of a simulated ADL should allow us to better estimate the in vivo biomechanical contribution of each structure. As expected, our preliminary selective cutting experiments in the ovine model indicated that the bony contact and menisci serve are the major restraints to joint compression during stance, and the ACL and MCL are the primary restraints to joint flexion and distraction during swing. These preliminary investigations led us to formulate the following hypotheses:

**Hypothesis 5:** Each ovine structure will restrain the same direction(s) of joint motion as the same structure in the human knee.

**Hypothesis 6:** Applying a simulated in vivo ovine motion will result in the bony contact and menisci serving as the major restraints during stance, and the ACL and MCL serving as the major restraints during swing.

For the next study (Chapter 4), we performed in vivo investigations of the ovine model. The overall objective of this project was to directly record in vivo ovine stifle joint kinematics for various ADLs, and then simulate and apply the subject-specific motions to each joint in vitro to measure the corresponding ACL forces and moments. For our preliminary in vivo experiment, our primary objective was to determine if surgically implanting knee motion sensors and an ACL force sensor would influence ovine gait. The following acceptance criterion was established based on earlier studies using force transducers in large animal models [158, 159, 203]: *post-surgical gait would only be deemed normal if*
Vertical ground reaction forces (VGRFs) remained greater than 80% of pre-surgery magnitudes. Another objective was to determine the effect of increasing speed and grade on ovine gait before and after surgery. The results of our earlier trials led us to formulate the following hypotheses:

**Hypothesis 7**: Surgical implantation of sensors will significantly decrease vertical ground reaction forces (VGRFs) in the operated limb; however the post-op values will be within 80% of pre-op values.

**Hypothesis 8**: Surgical implantation of sensors will significantly decrease single limb stance duration for the operated limb.

**Hypothesis 9**: Increasing treadmill speed will increase VGRFs pre- and post-operatively in the fore and hind limbs.

**Hypothesis 10**: Increasing treadmill grade will increase the hind limb VGRFs pre- and post-operatively by shifting the animal’s center of gravity in a posterior direction.

Chapter 5 discusses our achievements and challenges as we sought to simultaneously measure VGRFs, knee kinematics and AIFP voltages. While we were able to simultaneously record *in vivo* joint kinematics using an electromagnetic tracking system and the voltage outputs from the arthroscopically implantable force probe (AIFP) in the ACL, these two measurements presented many challenges. We were subsequently able to reproduce the *in vivo* motions using a 6 DOF robot. However, we noticed that due to motion recording error, the joint could not be oriented to match the full 6 DOF starting position of the gait path without damaging the joint. Also, the AIFPs frequently failed during *in vivo* testing due to durability issues. The strategies designed to overcome these challenges are also presented in Chapter 5.

The final manuscript (Chapter 6) bridges the *in vivo* and *in vitro* portions of this project as we sought to investigate the effect of perturbing (adjusting the starting point of) a simulated *in vivo* motion on intact knee and ACL forces. Our research goal was to measure *in vivo* knee motions and later recreate these physiologic motions using a 6 degree of freedom robot to measure joint and ACL forces.
However, there are motion recording and motion recreation errors associated with our methodology. Depending on the position along the load-displacement curve where different structures are working, small positional errors can have a large effect on joint forces and moments. Thus, the objective of this study was to determine how perturbing an ovine motion in each degree of freedom affects the joint and ACL kinetics. The results of our laboratory’s previous investigations using large preclinical animal models [158, 159, 203] indicated that knee structures can achieve up to 40% of failure strength for ADLs [158, 203] while the ACL functions at less than 10% of its failure strength [158]. Based on these results, we formulated the following hypotheses:

**Hypothesis 11**: Small translational or rotational perturbations in the motion would significantly affect the intact knee forces and moments.

**Hypothesis 12**: Small translational or rotational perturbations in the motion would not significantly affect ACL forces and moments.

The long term goal of this research is to use this methodology to establish functional tissue engineering parameters (FTEPs) to evaluate existing orthopaedic treatments and to design more effective treatments [91]. The clinical goal is to improve treatment outcomes by restoring normal joint function and thereby reducing the joint degeneration caused by knee injuries. The aims of this project sought to characterize a preclinical animal model for the human knee and to establish a methodology which investigators can use to establish FTEPs.

### 1.11 Specific Aims of Proposed Work

With the high prevalence of osteoarthritis following knee injury and the inability of current treatments to reduce the long term complications, our goal is to design more effective orthopaedic treatments. Although investigators have previously measured the full 6 DOF kinematics of human knee joints during walking [32], direct measurement of the corresponding 3D joint kinetics remains a challenge because the joint forces and moments of the knee cannot be directly measured *in vivo*. 
Investigators have attempted to measure joint forces and moments \textit{in vitro} by applying simulated clinical tests or artificial zero load/moment motion paths. Knowledge of the joint forces and moments for various activities of daily living (ADLs) is crucial to understand normal knee function, determine the effectiveness of treatments, and design more effective treatments.

Our lab has been seeking functional tissue engineering parameters (FTEPs) which can be used to establish design criteria and evaluation benchmarks for surgical therapies [91, 144]. Our preliminary goal is to characterize a preclinical animal model for knee biomechanics research and to develop a methodology using this model which can be used to measure joint kinetics for ADLs. This novel methodology will allow us to establish FTEPs based on subject-specific ADLs, rather than non-physiologic or average motions. This animal model can then be used to establish FTEPs based on the normal knee, investigate the effects of knee injuries, and to design and evaluate treatments.

The \textit{in vivo} and \textit{in vitro} experiments of this project combine to provide a novel research platform to investigate \textit{in vivo} knee joint biomechanics in an animal model. During \textit{in vivo} experiments, we simultaneously measure the vertical ground reaction forces, knee joint kinematics, and AIFP output during ADLs. The \textit{in vitro} studies investigate aspects of the novel robot methodology. This methodology requires simulating the 6 DOF \textit{in vivo} kinematics of each joint to measure the corresponding joint forces and moments. This technique will provide subject-specific force and moment measures which more closely represent the \textit{in vivo} kinetics of the joint.

In summary, this dissertation is composed of seven specific aims. For the first three aims, human and ovine \textit{in vivo} motions were applied to human knees and ovine stifles joints to measure the intact joint and ACL kinetics (3D forces and moments). A simulated \textit{ovine in vivo} motion was applied to both human and ovine joints to:

\textbf{Aim 1}: Determine if the intact joint and ACL kinetics were significantly different between species for the same applied motion.
Next, a simulated ovine *in vivo* motion was applied to ovine stifle joints and a simulated human *in vivo* motion was applied to human knees to:

**Aim 2:** Determine if the intact joint and ACL kinetics were significantly different between species when subjected to species-specific motions.

Third, a simulated ovine *in vivo* motion was applied to ovine stifle joints and a selective cutting procedure was implemented to:

**Aim 3:** Determine the primary and secondary restraining structures in the ovine stifle joint.

Ultimately, the results from these *in vitro* investigations of ovine stifle and human knee kinetics were used to assess the suitability of the ovine model as a biomechanical surrogate for the human knee. The next two aims focus on the *in vivo* experiments using the ovine model. Motion tracking and force sensors were implanted into the ovine stifle joint and an instrumented treadmill was used to measure vertical ground reaction forces (VGRFs) pre and post implantation. The study was designed to:

**Aim 4:** Determine the effect of surgically implanting sensors on average VGRFs, peak VGRFs, and single limb stance durations.

**Aim 5:** Determine the effect of increasing treadmill speed and grade on average VGRFs, peak VGRFs, and single limb stance durations before and after surgery.

The final two aims focus on validating our methodology which applies *in vivo* motions to measure joint and ACL kinetics. A simulated ovine *in vivo* motion was perturbed in each degree of freedom and applied to ovine joints to:

**Aim 6:** Determine the effect of motion recording and recreation errors on the force and moment measurements for the intact ovine stifle joint and ovine ACL.
**Aim 7:** Determine where in the ACL force-displacement curve the ACL operates during a simulated activity of daily living.

*Note:* Some ovine joints were used across the studies presented in Chapters 2, 3, and 6.
Patients frequently experience injuries to the soft tissues of the knee, particularly the ACL. Current ACL reconstruction strategies do not restore the native biomechanics of the knee which can lead to osteoarthritis in the long term. To design more effective treatments, investigators must first understand normal in vivo knee function for multiple activities of daily living (ADLs). Measuring the 6 degree of freedom kinetics of the knee during ADLs requires an animal model. The objective of this study is to determine if the ovine stifle joint is a suitable biomechanical model for the human knee. Simulated human and ovine in vivo motions were applied to human knee and ovine stifle joints to compare the intact joint and ACL kinetics between species. Only the compression-distraction forces significantly differed between the intact human and ovine joints. There were few ACL kinetic differences between species. During stance, applying the same ovine motion to both the human and ovine ACLs produced significantly different forces and moments between species. However, the ACL kinetics did not significantly differ between species during swing. Thus, the ovine stifle joint is a promising biomechanical surrogate for the human knee.

2.1 Introduction

Osteoarthritis is a prevalent clinical problem following ACL injury, even after surgical treatment [14, 30, 205]. The chronic degeneration of the knee joint has been attributed to the failure of current ACL reconstructions to restore the native joint biomechanics [20]. The ACL serves as the primary restraint to anterior tibial translation and also exhibits 3D forces and moments during daily activities [41]. Following ACL reconstruction, surgeons currently use clinical laxity tests such as the anterior
drawer and Lachman tests to determine the reconstruction efficacy. While these clinical tests can at best quantify the relative restoration of anterior tibial translation, these tests do not address changes in other degrees of freedom (DOF) of knee laxity. Additionally, ACL reconstructions have been shown to not restore rotational stability in the knee joint [21, 22]. These abnormal knee motions cause a shift in joint contact regions, causing wear of the articular cartilage and leading to an early onset of osteoarthritis [20].

In order to reduce the progression of joint degeneration, surgeons must restore normal knee function following ACL tears. To design more effective treatments, investigators must first understand normal in vivo knee biomechanics for multiple activities of daily living (ADLs). The 3D in vivo forces and moments of the normal intact knee, as well as those for just the ACL have not yet been determined for any ADL. Walking is the most common dynamic ADL in the general population. While knee kinematics have been measured in humans during walking [7, 32, 64], the joint and tissue forces are impossible to directly measure in vivo. Groups have attempted to measure tissue forces in the human knee using force and displacement sensors, however, the magnitudes reported were estimates because sensor calibration requires harvesting the joint and subjecting it to known loads. Investigators have also calculated the joint forces and moments using other techniques. The joint contact forces have been estimated from inverse dynamics calculations using kinematic and ground reaction force measurements [206, 207], as well as knee models which included muscle contributions [195, 208]. One group has measured in vivo joint contact forces in knee arthroplasty patients using an instrumented total knee replacement [209]. However, these results may not reflect normal joint loading in a healthy population. An alternative is to use an animal model to measure in vivo joint forces and moments for multiple ADLs.

The objective of this study was to determine if the ovine stifle joint, which is anatomically similar to the human knee and a valid surgical model for the human cruciate ligaments [145, 155], is also a suitable biomechanical model for the human knee. The ovine stifle joint allows for 1) sensors or marker
systems to be rigidly fixed to the knee joint to accurately measure the 6 degree of freedom (DOF) kinematics, and for 2) the kinematics to be simulated and applied to the harvested limb to measure the corresponding joint forces and moments. The 3D kinetics of the normal human knee or ovine stifle joint during an in vivo motion have not been previously reported. This information is needed to determine if the ovine model is a suitable biomechanical surrogate for the human knee.

In this study, we applied simulated human and ovine in vivo motions to human knees and ovine stifle joints to compare the joint and ACL function between species. We measured and compared the intact joint and ACL forces and moments for 3 cases: a simulated in vivo human walking motion applied to human knee joints (Human-Human), a simulated in vivo ovine walking motion applied to human knee joints (Ovine-Human), and a simulated in vivo ovine walking motion applied to ovine stifle joints (Ovine-Ovine). We formulated the following hypotheses: 1) The ovine joint will exhibit higher force and moment magnitudes than the human knee for the ovine motion since it is a stiffer joint than the human knee; 2) However, the similar anatomy of the human knee and ovine stifle joint will produce similar joint force and moment patterns when the same ovine motion is applied across species; 3) The human joint and ACL will exhibit higher forces and moments when subjected to the human motion, due to the larger range of motion compared to the ovine motion; 4) The ovine ACL will exhibit higher forces than the human ACL when the same ovine motion is applied across species. The goal of this study was to characterize a potential biomechanical model for the human knee. If the ovine is an acceptable model, the ovine stifle joint will be used to establish design criteria and evaluation benchmarks for traditional and tissue engineered ACL reconstructions.

2.2 Design & Methods

Experimental Design. Seven ovine stifle joints (no pairs) and six human knees (no pairs) were tested using a 6 DOF robot. A Kuka robot (KR210; Kuka Robotics Corp., Clinton Township, MI) equipped with a 6-axis load cell (Theta Model; ATI Industrial Automation, Apex, NC) was used to reproduce a 6
degree of freedom (DOF) ovine in vivo walking motion [146] and a 6 DOF human in vivo walking motion [32]. Each ovine stifle joint was subjected to 10 cycles of the ovine gait path and each human knee was subjected to 10 cycles of the ovine gait path and 10 cycles of the human gait path [32] while recording the 3D joint forces and moments. The bony interaction and all soft tissue except the ACL were removed from each joint, leaving the ACL as the only structure transmitting force and moments across the joint. The testing procedure was then repeated for the ACL condition to acquire 3D ACL forces and moments. We analyzed the intact joint and ACL forces and moments to determine if the anatomical similarities between the human knee and ovine stifle joint translate into functional similarities during in vivo motions.

**Joint Preparation.** Human cadaveric lower limbs (4 female, 2 male, age: 83 ± 4 (SEM) yrs) and ovine hind limbs from skeletally mature, mixed-breed female sheep (3–4 yrs; 50-78 kg) were stored at -20°C until the day prior to testing. All human knees and ovine stifle joints were screened for soft tissue injuries. The detailed joint preparation procedure, including the use of a custom tibial fixture to establish the tibial joint coordinate system [198] has been reported previously [153]. The limbs from both species were prepared similarly; each limb was dissected free of all muscles and tendons, leaving the knee joint capsule, the collateral and cruciate ligaments, and both menisci intact. A small longitudinal incision was made in the center of the posterior capsule to access the posterior attachments of the intracapsular structures. The joints were wrapped in saline-soaked gauze and hydrated periodically throughout the preparation and setup procedures. The femur was left intact and the distal half of the tibia was removed. The proximal half of the tibia was secured in a specially-designed fixture with bone cement.

The tibial fixture was used to establish the tibial joint coordinate system. First, the Y-axis was established along the medial-lateral (M-L) axis of the tibia by drilling a steel rod through the tibia just distal to the insertions of the MCL and LCL so that the axis was parallel to the relatively flat, proximal
tibial surface. The Z-axis was then aligned along the compression-distraction (C-D) axis of the tibia by placing a pipe around the tibial shaft and orienting the central axis of the pipe parallel to the long axis of the tibia. With the Y and Z axes established, two pins (diameter = 2.8 mm) were drilled through two sets of holes in the pipe orthogonal to each other and the pipe was filled with polymethyl methacrylate (PMMA) to secure the tibia within the pipe. Two identical pins were also drilled orthogonal to each other in the central portion of the femoral epiphysis and a cylindrical mold (4 cm diameter, 4 cm long) was placed over the pins and filled with PMMA to prevent motion of the bone inside the bone cement. After the PMMA cured and the mold was removed, the remaining PMMA cylinder mated with the femoral fixture.

**Robot Setup.** The tibial fixture was attached to the robot and aligned to the robot axes, and the tibial joint center point (JCP) on the tibia was digitized using a 3D coordinate measurement machine (CMM, Faro Digitizer Model #F04L2; FARO Technologies Inc., Lake Mary, FL). We first attached the tibial fixture to the load cell on the robot and adjusted the fixture to align the tibial joint coordinate system with the robot and load cell axes. A point on the tibial ACL insertion site (between the tibial spines) was digitized as our tibial JCP. We then input the 3D tibial JCP coordinates into our robot software and load cell software so that all rotations and translations were imposed about this point and all forces and moments were recorded about this point.

For the ovine motion, we attached the femur to the base fixture and adjusted the joint position to the starting position for the ovine motion. The robot arm was adjusted to guide the hanging femur onto the femoral base fixture. We then zeroed the forces and moments prior to securing the femur to the base fixture. The flexion angle was measured with the CMM and adjusted to midswing (60.5°), where small translations were used to minimize the forces and moments to <5 N and <1 Nm, respectively. This flexion angle was selected as our ovine starting point because we assumed that the
joint is minimally loaded at the midpoint of joint flexion since there is an absence of ground reaction forces.

For the human motion, we attached the femur to the base fixture and adjusted the joint position to the starting position for human motion. The robot arm was adjusted to guide the hanging femur onto the femoral base fixture. We then zeroed the forces and moments prior to securing the femur to the base fixture. The flexion angle was measured with the CMM and adjusted to 45°, where small translations were used to minimize the forces and moments to <5 N and <1 Nm, respectively. A portion of the human gait path was applied to move the joint to 20° of flexion, which corresponds to the maximum flexion angle during stance of the human gait path. At this flexion angle, the anatomical points required to establish the tibial and femoral joint coordinate systems [198] were marked. These points were then digitized and the 3D coordinates were input into a custom MATLAB program which output the 6 DOF position of the tibial JCP with respect to the femoral JCP and calculated the 3 rotations required to match the rotations reported by Lafortune et al. [32] at midstance. The robot was adjusted to achieve these rotations. This process was repeated until each rotational position was validated to within ± 0.5°. Subsequently, 500 N of force in compression was applied to the joint. This magnitude was determined from ground reaction forces and kinematic data acquired by Hewett et al. (data not published). We then applied 10 cycles of the human gait path, which reduced the force in compression. The process of applying 500 N was repeated until the joint maintained a compressive force of 500 (± 10) N after 10 cycles. The femoral and tibial JCPs were then digitized again and the robot was adjusted to achieve the anterior-posterior and medial-lateral displacements reported by Lafortune et al. [32].

**Robot Testing.** The human and ovine joints were subjected to separate testing procedures. All tests were performed at room temperature. The ovine joints were only subjected to the ovine gait path, whereas the human joints were subjected to both the ovine and human gait paths. The human gait path was not applied to the ovine joint because the large range of motion would damage the ovine joint.
Once at the ovine starting position, the ovine gait path was applied to the ovine stifle joint. We applied the first set of 10 cycles of the ovine gait path to precondition the joint, followed by another 10 cycles to record the forces and moments for the intact joint. We then removed all soft tissue and the distal portion of the femoral condyles, leaving the ACL as the only structure transmitting force across the joint. The 10 cycles were applied again to record forces and moments for the ACL. Finally, the ACL was removed and another 10 cycles were applied to record the forces and moments for the bone-only condition.

The human joint test method was similar to that of the ovine joint, except the human joint was subjected to two different simulated motions, each with a different starting position. At the ovine starting position, we applied the first set of 10 cycles of the ovine gait path to precondition the joint, followed by another 10 cycles to record the forces and moments for the intact joint. We then adjusted the robot position to the starting position of the human motion, where we applied 10 cycles of the simulated human gait path to precondition the joint, followed by another 10 cycles to measure forces and moments for the intact human knee. We then removed all soft tissue and the distal portion of the femoral condyles to attain the ACL condition. Another 10 cycles of the human gait path were again applied to record the ACL forces and moments. We then adjusted the robot position to the ovine starting position and applied 10 cycles of the ovine gait path to record the human ACL forces and moments for the ovine motion. Finally, the ACL was removed and the forces and moments were recorded for the bone-only conditions of both the ovine and human gait paths.

**Data Analysis.** The forces and moments recorded for the bone-only condition were subtracted from joint and ACL force and moment measurements to eliminate the effect of the tibial weight and robot inertia on force and moment measurements. The 8th and 9th cycles of each 10 cycle test were used for analysis to minimize any viscoelastic effects. For the intact knee and ACL conditions of both species, we examined the forces along and the moments about the 3 anatomical axes. Forces were
reported with respect to the tibial joint coordinate system (TJCS) [198] along the anterior-posterior (A-P), medial-lateral (M-L), and compression-distraction (C-D) axes. Moments were also reported with respect to the TJCS, with abduction-adduction (Ab-Ad), flexion-extension (F-E), and internal-external (I-E) moments acting about the A-P, M-L, and C-D axes, respectively. Gait cycles were normalized (%) to consecutive heel strikes, and forces and moments were averaged across subjects for the intact joint and ACL conditions. All forces and moments are reported for both the stance and swing phases of gait.

**Statistical Analysis.** All force and moment data was normal and heteroscedastic. For both the intact and ACL conditions, a two-way repeated measures analysis of variance (ANOVA) was performed for the stance and swing phases across all directions of force and moment with the motion and limb as fixed factors. Post hoc comparisons were performed to compare the 3 test cases (Human-Human, Ovine-Human, Ovine-Ovine) using a Tamhane correction. The significance level for all comparisons was set at $p < 0.05$.

### 2.3 Results

**Intact Joint.** During both stance and swing, only C-D intact knee forces significantly differed between cases. The A-P and M-L forces, and all directions of moments did not differ between cases. During stance, the only significant difference was the larger compression force exhibited by the Human-Human case compared to the Ovine-Human case ($p < 0.016$) and Ovine-Ovine case ($p < 0.001$); Fig. 5. Similarly, during swing, the Human-Human case exhibited a significantly larger compression force than the Ovine-Human ($p < 0.017$) and Ovine-Ovine ($p < 0.010$) cases; Fig. 5.
Figure 5  Distraction-compression force in the intact knee during stance (left) and swing (right). The Human-Human case (N=6) exhibited greater joint compression forces than the Ovine-Human (N=6) and Ovine-Ovine (N=7) cases throughout the gait cycle.

ACL. During stance, ACL A-P and M-L forces, and Ab-Ad and F-E moments differed between the Ovine-Ovine and Ovine-Human cases, whereas there were no differences during swing. The ovine ACL exhibited significantly larger anterior (p<0.022; Fig. 6) and medial (p<0.034; Fig. 7) forces than the human ACL subjected to the same ovine motion. The ovine ACL subjected to the ovine motion also exhibited significantly larger adduction (p<0.021) and flexion moments (p<0.028) than the human ACL subjected to the same ovine motion. ACL C-D forces (Fig. 8) and I-E moments did not differ between cases during stance. During swing, there were no significant force or moment differences between cases; Fig. 6 - 8.
Figure 6  Anterior-posterior (A-P) ACL force during stance (left) and swing (right). A-P forces significantly differed between the Ovine-Human (N=6) and Ovine-Ovine (N=7) cases during stance, but there was no difference between cases during swing.

Figure 7  Medial-lateral (M-L) ACL force during stance (left) and swing (right). M-L forces significantly differed between the Ovine-Human (N=6) and Ovine-Ovine (N=7) cases during stance, but there was no difference between cases during swing.

Figure 8  Distraction-compression (C-D) ACL force during stance (left) and swing (right). C-D forces did not differ between cases during stance or swing.
2.4 Discussion

The force and moment differences were minimal between the three cases (Human-Human, Ovine-Human, Ovine-Ovine) for both the intact knee and ACL. The only significant difference between the joint kinetics of the intact human knee and ovine stifle joint was the C-D force, as the Human-Human case exhibited significantly larger compression force than both the Ovine-Human and Ovine-Ovine cases during stance and swing. However, the 500 N of compression force applied to the human joint for the human motion but not for the ovine motion produced the difference. During stance, the ACL kinetics differed between species for the same motion primarily because the ovine motion failed to load the human ACL while the ACL remained loaded for the other two cases (Human-Human, Ovine-Ovine). During swing, there was no difference in ACL kinetics between cases. The ovine and human ACL restraining roles were similar to those previously reported for the human ACL subjected to simulations of clinical tests [40-43, 105, 106, 108, 123].

ACL forces and moments only differed during stance and only between the Ovine-Human and Ovine-Ovine cases. During stance, the kinetic differences between the human and ovine ACL when subjected to the same applied ovine motion is attributed to the ovine motion’s small range of motion failing to load the human ACL, which is accustomed to restraining a larger human range of motion. While it may seem that the Human-Human (Fig. 6 and 7) case also exhibits significantly greater force than the Ovine-Human case, there was no significant difference between the two cases. This was due to the large variance in ACL force between subjects as some cadaveric ACLs exhibited minimal ACL forces throughout the human motion.

The restraining roles of the human ACL and ovine ACL are similar and coincide with the previously reported roles of the human ACL [40-43, 105, 106, 108, 123]. The human ACL and ovine ACL restrained the same directions of displacements and rotations during stance and swing. Across all cases, both the human and ovine ACLs restrained anterior, medial, and distraction displacements, as well as
adduction and flexion rotations. These roles are similar to the roles previously reported for the human
ACL which indicated that the ACL serves as a major restraint to anterior displacement [41, 105, 106] and
a secondary restraint to medial displacement and flexion, abduction/adduction and internal rotations
[40, 42, 43, 108, 123].

The joint compression forces are comparable to previous estimates. Prior to this study, joint
kinetics had not been reported for the ovine stifle joint. Previously, only joint contact forces have been
estimated for healthy human knee joints. Our peak compression forces are less than previous estimates
of human tibiofemoral contact forces. Previous studies estimated joint compression forces of the
normal human knee joint during level walking to be 2 to 3 times body weight, with some estimates as
high as 6 times body weight [207, 210, 211]. Our average human intact knee compression forces peaked
at 1232 ± 112 (SEM) N. While our peak compression force is lower than previous estimates, we had no
knowledge of the weight of our human subjects. Thus, we applied 500 N of compression force prior to
applying the human motion to the human knee joint based on average knee compression forces from
unpublished data acquired by Hewett et al. Thambyah et al. [206] calculated the average compression
force at midstance to be 779 N and peak compression magnitude to reach 2075 N, whereas we applied
only 500 N of compression force at midstance and reached a peak compression magnitude of only 1232
N. For future studies, we will investigate using a greater compression force at the starting position.
However, a limitation of this study is our use of older human cadaveric knees which exhibit reduced
material and structural properties compared to a young healthy subject. This may have caused our
compression force to be lower than previous estimates.

This study had several other limitations. First, the biomechanical interactions between joint
contact force and ACL forces cannot be determined using the stiffness-based testing methodology
implemented in this study. Although we assume that removal of the bony contact causes minimal
changes in ACL biomechanics, in the future we plan to investigate the effect of removing bony contact
on the position of the ACL insertion sites. Using the CMM, we will digitize the 3D coordinate of the ACL insertion sites before and after removal of the distal femoral condyles. Next, the method for applying the ovine motion differed from that used for the human motion. The ovine motion was initiated at mid-swing, where forces and moments were assumed minimal. The human motion was initiated at midstance, where 500 N was applied to the joint. For future investigations of the ovine joint, we can apply a strategy similar to that used for the human joint. As was previously determined for the human joint, we can estimate the ovine joint compression force at a specific instance of gait based on ground reaction force measurements and joint angles. This force can then be applied to the joint at the chosen starting position. The method for determining the starting position for each species also had limitations. We assumed a zero force and zero moment condition at the midpoint of flexion for the ovine stifle joint, whereas the joint may experience some load at this position due to muscle forces, inertia, and tibial weight. Next, the same compression force was applied to every human knee joint although the body weights for the cadavers were not controlled. Also, an average in vivo human motion and an average in vivo ovine motion were used in this study. Ideally, the subject-specific kinematics for each joint should be recorded in vivo and applied to the same joint since the joint morphology and biomechanics among subjects will vary. This would allow for the measurement of 3D kinetics that would be more indicative of in vivo forces and moments. Limitations of our robot methodology such as gait path reproduction speed (≈1/10th of in vivo speed) have been reported previously [153] and are discussed in Chapter 6.

These results suggest that although there are differences between the knee kinematics and joint morphology of the human knee and ovine model, the overall intact joint and ACL functions are similar between species as determined from joint and ACL kinetics. The kinetic measurements for the three cases suggest that the similar anatomy between the human knee and ovine stifle joint allow similar joint function as determined from joint forces and moments. Forces and moments exhibited by the ACL are also similar between species. Thus, the ovine stifle joint is a suitable model for the evaluation of
traditional and novel ACL treatment strategies. Furthermore, knee biomechanics investigations using in vivo knee kinematics recorded for multiple ADLs in the ovine model could provide valuable insight for better understanding the normal function of the human knee, as well as the effect of injury and repair. The next step will be to use the ovine model to establish design criteria and evaluation benchmarks for traditional and tissue engineered ACL reconstructions with the long term goal of minimizing joint degeneration after ACL injury.
Primary and Secondary Restraints in the Ovine Stifle Joint for a Simulated In Vivo Motion [212]

Knee soft tissue structures are frequently injured and osteoarthritis continues to be a clinical problem following treatment. Understanding the contribution of these structures to knee kinetics during activities of daily living (ADLs) is crucial to the development of more effective treatments. This study was designed to determine the restraining roles of the different knee structures during an ADL. We used a 6 degree of freedom robot to reproduce an in vivo walking motion while measuring 3D joint forces and moments in the ovine stifle joint. Using a selective cutting procedure, we determined the primary and secondary knee structures contributing to 3D knee forces and moments. The bony interaction and medial meniscus provided the major contribution during stance, whereas the MCL and ACL were the key contributors during swing phase. Comparisons to the previously reported contributions for the structures of the human knee indicated that the ovine stifle joint offers a suitable biomechanical surrogate for the human knee. This study contributes to our goal of determining functional tissue engineering parameters by characterizing a model that can provide crucial design criteria for the development of more effective treatments, thus reducing the incidence of osteoarthritis following knee injury.

3.1 Introduction

Soft tissues in the knee are frequently injured and joint degeneration continues to be a clinical problem following treatment. Severe knee injuries (e.g. ACL and meniscus tears) usually require costly surgical treatment and rehabilitation. However, patients continue to develop long term osteoarthritis even after surgical treatment [30, 205]. The early onset of osteoarthritis and increased susceptibility to
further injury following surgical treatment is attributed to the inability to restore the native knee kinematics using current procedures [20]. Further complicating the clinical problem is that our understanding of the normal knee biomechanics during actual activities of daily living (ADLs) is limited. Thus, surgeons are currently repairing soft tissue structures with limited knowledge of the function of these structures during ADLs.

The role of soft tissues and bone in the knee during gait is vital to injury prevention and treatment. While investigators have provided important information about knee kinematics during ADLs [32, 64], our current understanding of knee kinetics is usually limited to non-physiologic motions which simulate clinical examinations (e.g. anterior drawer test). The restraining roles of soft tissue structures have only been determined for human cadaveric knees using laxity or stiffness based tests combined with selective cutting procedures [41, 199]. Thus, our knowledge of how the knee and its structures function during ADLs is limited. In addition, our inability to functionally “isolate” the knee or its structures and to impose realistic physiologic motion patterns during calibration in human subjects forces investigators to pursue kinetic measurements in animal models.

*In vivo* knee motions can now be measured and applied to the harvested joint to measure the corresponding forces and moments in the intact knee and for individual structures. By characterizing the function of the intact knee and the individual structures, we can evaluate the treatment strategies not just on the whole joint level, but also determine if the repair has adversely affected individual structures, which could ultimately lead to long term joint degeneration. The ovine model is a viable candidate for these studies because the ovine stifle joint is structurally and biomechanically similar to the human knee [145, 155] and is a suitable surgical model for the human knee and cruciate ligaments [155].

In this comprehensive biomechanics study, we apply a simulated *in vivo* ovine motion to ovine stifle joints to investigate the restraining roles of knee structures. The objective of this study is to
determine the restraining roles of the different knee structures during a simulated ADL. We hypothesize that the primary and secondary contributors to each direction of joint forces and moments for the ovine stifle joint will be similar to those previously reported for the human knee. Our long term research goal is to measure functional tissue engineering parameters during ADLs to provide design criteria for more effective strategies to repair or reconstruct these structures.

3.2 Design & Methods

Experimental Design. Three left and six right hind limbs (no pairs) from skeletally-mature, mixed breed female sheep (3–4 yrs old; 50-78 kg) were tested using a 6 DOF robot (KR210; Kuka Robotics Corp., Clinton Township, MI) equipped with a 6-axis load cell (Theta Model; ATI Industrial Automation, Apex, NC). The ovine stifle joint is anatomically and biomechanically similar to the human knee [145, 155]. A robot was used to reproduce a 6 degree of freedom (DOF) ovine in vivo motion adapted from Tapper et al. [146]. The intact knee joint was subjected to 10 cycles of this gait path while joint forces and moments were recorded. A selective cutting sequence was implemented in which a structure (e.g. MCL) was cut and the same 10 cycle gait path was applied to record joint forces and moments. The cutting sequence was randomly ordered between subjects to reduce the effect of cutting order based on tissue interactions. The only exception was that the sequence always concluded with the elimination of bony interaction, followed by the removal of the ACL. Since our lab is primarily concerned with joint and ACL biomechanics, the ACL-isolated condition allowed us to measure ACL forces and moments as the ACL was the only structure transmitting force across the joint. This study permitted us to determine the primary and secondary structures contributing to 3D knee joint forces and moments and to compare the restraining roles of each ovine structure to the previously reported roles of the same structure in the human knee.
**Sample Preparation and Setup in Robot.** Each limb was prepared and secured to the robot fixtures according to the previously reported procedure [153] described in Chapter 2. Ovine limbs were obtained from a local vendor and stored at -20°C until the evening before testing. Each limb was dissected free of all muscles and tendons, leaving the knee joint capsule, the collateral and cruciate ligaments, and both menisci intact. The tibial fixture was rigidly attached to the load cell on the 6 DOF robot and adjusted to align the tibial joint coordinate system [198] with the robot and load cell axes.

The tibial joint center point (TJCP) was then digitized and the femur was secured. Using a coordinate measurement machine (CMM, Faro Digitizer F04L2, FARO Technologies Inc., Lake Mary, FL), we selected the ACL insertion site on the tibia (between the tibial spines) as the TJCP. This TJCP was entered as the tool center point for the robot and load cell so that all rotations and translations were imposed about this point and all forces and moments were recorded about this point. We then attached the femur to the base fixture and zeroed the forces and moments. The flexion angle was measured with the CMM and the flexion angle was adjusted to 60.5°, the midpoint of joint flexion during swing phase. This was selected as the starting flexion angle because it corresponds to where minimal forces and moments would be expected. Small translations were used to minimize the forces and moments to <5 N and <1 Nm, respectively. This was our starting position.

**Simulated 6 DOF In Vivo Motion Robot Testing.** Once at the starting position, the ovine gait path was applied to the ovine stifle joint. All tests were performed at room temperature. We applied the first set of 10 cycles (8.22 sec cycle duration) of the ovine gait path to precondition the joint followed by another 10 cycles to record the forces and moments for the intact joint. We then cut a structure (e.g. MCL) and applied another 10 cycles while recording the forces and moments. This process was continued as each soft tissue structure (MCL, LCL, PCL, medial meniscus, lateral meniscus, medial capsule and lateral capsule) was removed in random order. The ACL was always the final soft tissue structure remaining. Prior to removing the ACL, the femoral condyles were removed so that only
the ACL spanned the joint. Finally, the ACL was removed, and the forces and moments were recorded for the remaining tibia and femur.

**Data Analysis.** We determined the contribution of each structure to joint kinetics by computing the difference in forces and moments due to the removal of the structure. The removal-induced changes in 3D forces and moments for each structure were calculated and reported as the structure’s contributions. The final “bone-only” condition was subtracted from all measurements to account for the biasing of the load cell at a single point and robot inertia. The 8th and 9th cycles of each 10 cycle test were used for analysis to minimize any viscoelastic effects. Data were averaged across animals throughout a normalized gait cycle (%) for the intact joint and each structure. Forces were reported with respect to the tibial joint coordinate system (TJCS) \[198\] along the anterior/posterior (A-P), medial/lateral (M-L), and compression/distraction (C-D) axes. An anterior force contribution denotes that the structure resists an anteriorly-directed translation. Moments were also reported with respect to the TJCS. Abduction/adduction (Ab-Ad), flexion/extension (F-E), and internal/external (I-E) moments were about the A-P, M-L, and C-D axes, respectively. A flexion moment contribution denotes that the structure resists a flexion rotation.

**Statistical Analysis.** For each degree of freedom, the average force and moment was calculated for each structure during stance and swing. A one-way repeated measures analysis of variance (ANOVA) was performed to determine force/moment differences for the different structures during stance and swing. In addition, the forces/moment contributions were analyzed at the points of interest during gait (e.g heel-strike, midstance, toe-off, peak flexion) using a one-way ANOVA. All data was normal and homoscedastic. Post hoc comparisons were made using the Bonferroni method. The significance level for all comparisons was set at \( p < 0.05 \).
3.3 Results

Stance Phase. The bony interaction served as the major restraining structure during stance. The bony interaction and medial meniscus were the primary and secondary contributors to the net posterior intact knee force, respectively, while the PCL also contributed to posterior forces at midstance. The ACL provided the only significant anterior force throughout stance (Fig. 9). The bony interaction was the primary contributor to the net medial intact knee force, while the ACL contributed at heel-strike and toe-off. The medial meniscus contributed a significant lateral restraining force (Fig. 10). The bony interaction and medial meniscus were the primary and secondary contributors to the net compressive intact knee force, respectively, while the lateral meniscus contributed at heel-strike and toe-off. The PCL contributed a distraction restraining force throughout stance. The bony interaction was the primary contributor to the net adduction intact knee moment, while the medial meniscus, LCL, and ACL were secondary contributors. The lateral meniscus provided a significant abduction moment throughout stance (Fig. 11). The bony interaction and medial meniscus were the primary and secondary contributors to the net extension intact knee moment, respectively, while the PCL also contributed to extension moments at midstance. The ACL provided a significant flexion moment throughout stance (Fig. 12). The bony interaction and medial meniscus were the primary and secondary contributors to the net internal intact knee moment, respectively. The lateral meniscus provided a significant external moment at heel-strike and toe-off. These results are summarized in Table 1.

Swing Phase. The MCL served as the major restraining structure during swing. The ACL and MCL were the primary and secondary contributors to the net anterior intact knee force, respectively. The bony interaction contributed a significant posterior force throughout swing, while the lateral meniscus contributed at peak flexion (Fig. 9). The MCL was the primary contributor to the net lateral intact knee force, while the bony interaction contributed at peak flexion. The ACL contributed a significant medial force throughout swing, while the lateral meniscus contributed at peak flexion (Fig.
The bony interaction was the primary contributor to the net compression intact knee force, while the lateral and medial menisci provided secondary contributions. The MCL contributed the predominant distraction force throughout swing, while the PCL and ACL also significantly contributed. The MCL and lateral meniscus were the primary and secondary contributors to the net abduction intact knee moment, respectively, while the bony interaction and medial capsule also contributed at peak flexion. The ACL and medial meniscus contributed significant adduction moments throughout swing (Fig. 11). The MCL and ACL were the primary and secondary contributors to the net flexion intact knee moment, respectively. The bony interaction and lateral meniscus contributed significant extension moments throughout swing (Fig. 12). The MCL was the primary contributor to the net external intact knee moment, while the lateral meniscus contributed at peak flexion. These results are summarized in Table 2.

<table>
<thead>
<tr>
<th>Direction</th>
<th>Avg Intact Joint Force/Moment</th>
<th>Primary Structure</th>
<th>Secondary Structure(s)</th>
<th>Structure(s) in Opposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/P</td>
<td>Posterior Bone</td>
<td>Medial Meniscus</td>
<td>ACL</td>
<td>PCL *</td>
</tr>
<tr>
<td>M/L</td>
<td>Medial Bone</td>
<td>-</td>
<td>Medial Meniscus</td>
<td>ACL * ~</td>
</tr>
<tr>
<td>C/D</td>
<td>Compression Bone</td>
<td>Medial Meniscus</td>
<td>PCL</td>
<td>Lateral Meniscus * ~</td>
</tr>
<tr>
<td>Ab/Ad</td>
<td>Adduction Bone</td>
<td>Medial Meniscus, LCL, ACL</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>F/E</td>
<td>Extension Bone</td>
<td>Medial Meniscus</td>
<td>ACL</td>
<td>PCL*</td>
</tr>
<tr>
<td>I/E</td>
<td>Internal Bone</td>
<td>Medial Meniscus</td>
<td>-</td>
<td>Lateral Meniscus * ~</td>
</tr>
</tbody>
</table>

Table 1 Primary, secondary, and opposing structures to each direction of intact joint force and moment during stance (* = Midstance, * = Heel-strike, ~ = Toe-off)
Table 2 The primary, secondary, and opposing structures to each direction of intact joint force and moment during swing (^ = Peak Flexion)

<table>
<thead>
<tr>
<th>Direction</th>
<th>Avg Intact Joint Force/Moment</th>
<th>Primary Structure</th>
<th>Secondary Structure(s)</th>
<th>Structure(s) in Opposition</th>
</tr>
</thead>
<tbody>
<tr>
<td>A/P</td>
<td>Anterior</td>
<td>ACL</td>
<td>MCL</td>
<td>Bone</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Lateral Meniscus ^</td>
</tr>
<tr>
<td>M/L</td>
<td>Lateral</td>
<td>MCL</td>
<td>-</td>
<td>ACL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bone ^</td>
<td>Lateral Meniscus ^</td>
</tr>
<tr>
<td>C/D</td>
<td>Compression</td>
<td>Bone</td>
<td>Medial Meniscus, Lateral Meniscus</td>
<td>MCL, PCL, ACL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ab/Ad</td>
<td>Abduction</td>
<td>MCL</td>
<td>Lateral Meniscus</td>
<td>ACL, Medial Meniscus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Bone ^, Medial Capsule ^</td>
<td>-</td>
</tr>
<tr>
<td>F/E</td>
<td>Flexion</td>
<td>MCL</td>
<td>ACL</td>
<td>Bone, Lateral Meniscus</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I/E</td>
<td>External</td>
<td>MCL</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Lateral Meniscus ^</td>
<td>-</td>
</tr>
</tbody>
</table>

Figure 9 Contributing structures to anterior-posterior forces (N = 9). Stance phase begins at 0 and the vertical dashed line separates the stance and swing phases.
Figure 10 Contributing structures to medial-lateral forces (N = 9). Stance phase begins at 0 and the vertical dashed line separates the stance and swing phases.

Figure 11 Contributing structures to abduction-adduction moments (N = 9). Stance phase begins at 0% and the vertical dashed line separates the stance and swing phases.
3.4 Discussion

The contributions of the ovine structures for a simulated 6 DOF *in vivo* motion were similar to the roles previously reported for the human structures. The bony interaction and medial meniscus provide the major contribution to the forces and moments of the intact ovine stifle joint while the joint is compressed during stance, whereas the MCL and ACL are the key structural contributors as the knee becomes distracted during swing. The bony interaction serves a crucial role in joint biomechanics. However, previous studies applied non-physiologic motions which minimized joint compression, thereby limiting the role of the bony interaction. Based on comparisons to previously reported results for the structures of the human knee, the ovine stifle joint and its structures offer a suitable biomechanical surrogate for the human knee and its structures. Both the ovine and human ACLs serve as major restraints to anterior tibial translation and also restrain medial translation, adduction and flexion. In both species, the medial meniscus restrains compression and adduction. The ovine and human MCLs
both restrain anterior and lateral translations, as well as abduction. The previously reported roles of the human structures were reported for non-physiologic motions, which simulated clinical examinations [40, 42-44, 49, 106, 201]. Thus, to provide a more direct comparison between species, we plan to investigate the contributions of the human structures for a simulated human activity of daily living (ADL).

While the bony interaction primarily resisted compression, the compressed state of the joint also allowed the bony interaction to function as the primary restraint to every direction of translation and rotation, roles which have not been reported for the human knee. Only a single study has investigated the contribution of bony interaction alone to forces and moments in the human knee joint. Sakane et al. [106] reported that the bony interaction in the human knee was a secondary restraint to anterior displacement. However, the investigators only applied an anterior load throughout a zero force and zero moment passive flexion path even though the human knee experiences considerable compression forces during gait. The ovine bony interaction continued to play a role during swing, serving as the primary restraint to compression throughout swing, and as a secondary restraint to lateral translation and abduction at peak flexion. The bony interaction also restrained posterior translation during swing.

The ovine ACL functions similarly to the human ACL in that both the ovine and human ACL serve as major restraints to anterior displacements [41, 105, 106] and also contribute significantly to medial forces, adduction moments, and flexion moments. The ovine ACL differed from the human ACL by contributing to extension moments but not contributing to internal or abduction moments [40, 42, 43, 108, 123].

The roles of the ovine menisci are similar to those previously reported for the human menisci. The primary function of the human and ovine menisci is to improve the congruency of the joint and thereby distribute the large joint compression forces over a greater contact area [88, 145]. Both the human and ovine menisci contribute to internal/external and abduction/adduction moments, as well as
posterior and compression forces [92, 93, 95, 105]. The ovine menisci differed by also contributing to medial/lateral forces and extension moments. Both the human and ovine medial meniscus restrains compression and adduction [92, 93, 95]. The ovine medial meniscus differs by restraining posterior rather than anterior translation, and also restraining lateral translations, extension and internal rotations. The ovine medial meniscus may have exhibited these additional contributions because we applied a physiologic motion which maintained joint compression throughout stance. There is a lack of information regarding the role of the human lateral meniscus, likely because the human lateral meniscus is more mobile than the medial meniscus and thus may perform a less significant restraining role [88]. The human lateral meniscus does restrain anterior tibial translation when subjected to combined abduction and internal/external moments [213] whereas the ovine lateral meniscus restraints posterior, medial and compression translations, as well as abduction, extension, and external rotations.

Both the human and ovine PCLs function as major restraints to posterior translation, however, the other roles of the PCL differ between species. The ovine PCL resists posterior translation similar to the human PCL [41, 48, 49, 105]. However, the ovine PCL serves as a secondary restraint to posterior translation, providing only 9% of posterior force of the human joint whereas the human PCL reportedly provides 95% of the posterior force of the human joint [41]. These human findings were based on experiments using an uncompressed human knee joint. The ovine PCL differs from the human PCL as it functions as a major restraint to joint distraction and extension, and does not restrain lateral translation, abduction/adduction, and internal/external rotations like the human PCL [40, 43, 48, 49, 108].

The contributions of the human and ovine MCLs are similar but the human LCL serves a greater role than the ovine LCL. Both the human and ovine MCLs resist abduction [43, 105], while also restraining anterior and lateral translations [105, 106, 108]. The role of the ovine MCL differs by restraining joint distraction and flexion, as well as restraining external rotation rather than internal rotation like the human MCL. Both the human and ovine LCLs resist adduction [43, 105] but the human
LCL also resists external rotations [43, 49, 105, 214, 215], and to a lesser degree internal rotation, anterior translation and medial translations [43, 105, 108]. Although the LCL is oriented and positioned similarly between species [145], the ovine LCL may not contribute greatly during the simulated in vivo walking motion because it is attached to the thin and flexible ovine fibula, which is rudimentary and sometimes absent from the ovine joint [192]. Furthermore, excluding flexion/extension, the range of motions in the other 5 DOF were minimal and possibly not enough to challenge the LCL.

The differences between species can also be attributed to several other factors. First, the human contributions were determined from simulated clinical examinations whereas the ovine contributions were determined for a simulated in vivo walking motion. The clinical examinations may challenge the structure of interest more than an ADL. Unfortunately, the contributions of human knee structures have not been reported for an ADL. To determine the structural contributions for the human knee during an ADL, one would need to apply a 6 DOF human in vivo walking motion to the human knee joint. Although the ovine stifle joint contains all of the major soft tissue structures of the human knee [145, 155, 192], the human knee and ovine stifle joint have differences in morphology [145, 192] and in the native kinematics during a walking activity [32, 146]. Another limitation of our methodology includes the application of the simulated motion at ≈ 1/10th the actual in vivo speed. This limitation has been addressed previously [153] and is discussed in Chapter 6. Finally, the stiffness-based testing methodology allows for independence of cutting order. The cutting order was randomized between joints to eliminate any biomechanical effects of tissue interactions. However, the bony contact and ACL were always the second-to-last and last structures removed. In future studies, the position of the ACL insertion sites should be measured to determine the effect of bony contact removal on the ACL.

Understanding the contribution of the knee soft tissue structures and bony interaction to knee biomechanics is crucial to the development of better treatments for injury. This study contributes to our goal of determining functional tissue engineering parameters (FTEPs) [91] using a preclinical animal
model. Normal knee function during gait depends on the restraining roles of the soft tissue structures and bony interaction. While previous studies provide valuable information about the roles of knee structures, they have not presented critical information about their functions during actual ADLs. Knowledge of the contribution of each structure during ADLs will allow investigators to better understand the biomechanics of the knee joint and to establish FTEPs. These FTEPs will provide crucial design criteria for the development of more effective strategies for the prevention and treatment of knee injuries [91].
Activities of daily living (ADLs) generate complex, multidirectional forces in the anterior cruciate ligament (ACL). While calibration problems preclude direct measurement in patients, ACL forces can conceivably be measured in animals after technical challenges are overcome. For example, motion and force sensors can be implanted in the animal but investigators must determine the extent to which these sensors and surgery affect normal gait. Our objectives in this study were to determine (1) if surgically implanting knee motion sensors and an ACL force sensor significantly alter normal ovine gait and (2) how increasing gait speed and grade on a treadmill affect ovine gait before and after surgery.

Ten skeletally mature, female sheep were used to test four hypotheses: (1) surgical implantation of sensors would significantly decrease average and peak vertical ground reaction forces (VGRFs) in the operated limb, (2) surgical implantation would significantly decrease single limb stance duration for the operated limb, (3) increasing treadmill speed would increase VGRFs pre- and post operatively, and (4) increasing treadmill grade would increase the hind limb VGRFs pre- and post operatively. An instrumented treadmill with two force plates was used to record fore and hind limb VGRFs during four combinations of two speeds (1.0 m/s and 1.3 m/s) and two grades (0 deg and 6 deg). Sensor implantation decreased average and peak VGRFs less than 10% and 20%, respectively, across all combinations of speed and grade. Sensor implantation significantly decreased the single limb stance duration in the operated hind limb during inclined walking at 1.3 m/s but had no effect on single limb stance duration in the operated limb during other activities. Increasing treadmill speed increased hind limb peak (but not average) VGRFs before surgery and peak VGRF only in the unoperated hind limb during level walking after surgery. Increasing treadmill grade (at 1 m/s) significantly increased hind limb
average and peak VGRFs before surgery but increasing treadmill grade post op did not significantly affect any response measure. Since VGRF values exceeded 80% of pre op levels, we concluded that animal gait post op is near normal. Thus, we can assume normal gait when conducting experiments following sensor implantation. Ultimately, we seek to measure ACL forces for ADLs to provide design criteria and evaluation benchmarks for traditional and tissue engineered ACL repairs and reconstructions.

4.1 Introduction

Treating injuries to the anterior cruciate ligament (ACL) and its graft replacements remain important clinical and basic science problems. As many as 250,000 ACL are reported to occur each year [217, 218]. Such injuries can change knee kinematics and loading patterns in the surrounding structures. Left untreated, these changes can lead to joint degeneration and osteoarthritis [27, 218-223]. Surgeons reconstruct over half of the ruptured ligaments, often using tendon autografts [51, 52]. Early studies reported a success rate exceeding 90% after surgery [23-26, 224]. Despite these successes, investigators have also cited postoperative complications including donor site morbidity, patellar discomfort and joint laxity [25, 26, 127, 224-228]. In the intermediate term, these ACL reconstruction techniques do not restore normal 3-D knee kinematics [28, 29] and in the longer term, patients undergoing ACL reconstruction still have a high risk of radiographic osteoarthritis within 14 years following their injury [30]. Given the ongoing clinical concern of osteoarthritis, current ACL reconstruction strategies do not appear to be adequately addressing the long term complications.

Several compounding factors limit our understanding of the mechanisms responsible for these in vivo complications. During activities of daily living (ADLs), the ACL experiences three-dimensional forces throughout the range of knee motion, yet researchers normally load the knee in a single direction at selected knee positions before measuring reaction forces and deformations [106, 152, 229-235]. Using these more simplified testing methods, investigators have determined that the ACL serves as a
primary restraint to anterior tibial translation, a major secondary restraint to internal tibial rotation, and
a minor secondary restraint to external tibial rotation and varus-valgus angulations [29, 41, 236, 237].
While these studies are valuable, they do not provide critical information about ACL function during
normal ADLs. As a result, data is lacking regarding the complex nature of ACL forces, especially in
response to real or simulated in vivo loading conditions.

To improve long term outcome following ligament injury, investigators need to understand ACL
function during ADLs. Unfortunately, measuring knee motions during any in vivo activity has been
difficult in patients and directly recording the corresponding ACL forces has been impossible.
Investigators can now more accurately measure in vivo human knee motions during normal activities of
daily living [32, 64, 196, 238]. However, the inability to calibrate tissues implanted with force sensors
has prevented investigators from actually measuring in vivo knee forces in human patients. Henning et
al. [239] first attempted to quantify human in vivo ACL forces for different activities by implanting a
strain gauge sensor. However, they could only make these recordings in patients with partially torn
ACLs due to the invasiveness of sensor implantation and were unable to directly calibrate the forces.
Roberts et al. [240] later attempted to measure in vivo forces in the human ACL by implanting an
arthroscopically implantable force probe or AIFP (MicroStrain, Inc., Burlington, VT) in the anterio-medial
bundle of the ACL of a healthy adult volunteer. Unfortunately, these measurements could only be taken
during passive flexion/extension motions rather than for actual ADLs and calibration required that in
vitro data be obtained from other cadaveric knees. It is this inability to functionally “isolate” the
ligament and impose realistic physiologic loading patterns during calibration that has forced
investigators to pursue similar measurements in animal models.

The ovine model offers an attractive alternative for relating knee and ACL structure and
function. Investigators have carefully examined the anatomy of the ovine stifle joint and found it to be
both a valid surgical model of the human knee and cruciate ligaments [145, 155] and an ideal
experimental model for studying a range of orthopedic conditions and treatments [145]. Appleyard et al. [241, 242] used the ovine model to determine how meniscectomy alters articular cartilage biomechanics [241] and how ACL transection changes meniscal and articular cartilage function [242]. Tapper et al. [146] also characterized 3-D joint motion during walking, inclined walking, and trotting in the intact ovine stifle joint instrumented with a surgically-implanted rigid marker system. This group also determined vertical ground reaction force (VGRF) patterns for walking prior to and one and two days after implantation. They then placed the instrumented limb in a hexapod robot to simulate normal 3-D kinematics [243] which could potentially be used to determine joint or ligament forces. Although such methodologies are exciting and offer the prospects of better understanding ligament function for actual ADLs, 3-D kinematics must still be verified. Our challenge remains to accurately record and replicate joint motions to measure corresponding ACL forces in order to establish effective design requirements for new and existing treatment modalities.

Our group seeks to quantify functional tissue engineering parameters (FTEPs) for ADLs to more effectively repair damaged load-bearing tissues [244]. Critical among these FTEPs for all load-bearing tissues are realistic in vivo forces and displacements for different ADLs [144]. While our group has estimated rabbit and goat tendon forces and goat ligament forces for numerous ADLs [158, 159, 203, 245, 246], we could not precisely replicate in vivo joint positions during our in vitro calibration procedures. Others have even measured in vivo ligament displacements in the anterior region of the human ACL for selected activities [197, 247-249], but could neither compute tissue strains without knowing initial transducer length at zero tissue force nor infer tissue forces without ligament material properties. Joint kinematics were also not directly measured.

Given these gaps in knowledge, we sought to directly record in vivo 3-D kinematics in the ovine knee and then simulate these motions to measure ACL forces for different ADLs. Our primary objective was to determine if surgically implanting knee motion sensors and an ACL force sensor would influence
gait in the adult ovine model. We also sought to determine how increasing treadmill speed and grade would alter ovine gait before and after surgery. We hypothesized that 1) surgical implantation of sensors would significantly decrease average and peak VGRFs in the operated limb. However, VGRFs should maintain a minimum of 80% of pre op values to assume normal gait when measuring ACL force and knee kinematics using the implanted sensors. This criterion was selected based on previous experience inducing surgical treatments and implanting sensors in the stifle joint of large animals. We also hypothesized that 2) surgical implantation of sensors would significantly decrease single limb stance duration for the operated limb, 3) increasing treadmill speed would increase VGRFs pre and post operatively in the fore and hind limbs, and 4) increasing treadmill grade would increase the hind limb VGRFs pre and post operatively by shifting the animal’s center of gravity in a posterior direction. This research study is the first step in our longer-term goal of quantifying FTEPs to establish design criteria and evaluation benchmarks for surgical therapies [91, 144].

4.2 Design & Methods

Experimental Design. Ten skeletally-mature, female sheep (3–4 yrs old; 50-78 kg; Species: Suffolk; Vendor: Purdue University, West Lafayette, IN) were used. The ovine stifle joint is morphologically and biomechanically similar to the human knee [155]. The sheep knee is large enough to: 1) implant motion sensors on the medial aspect of the femur and tibia, 2) implant a force sensor in the ACL mid-substance, and 3) perform reproducible ligament reconstruction. Prior to implantation surgery, vertical ground reaction forces (VGRFs) were recorded while each sheep was subjected to daily combinations of two speeds (1.0 m/s and 1.3 m/s) and two grades (0° [level] and 6° [inclined]). We initially attempted a wider range of speeds and grades, but the sheep showed abnormal gait at speeds less than 1.0 m/s and after surgery, had difficulty maintaining traction at speeds and grades greater than 1.3 m/s and 6°, respectively. At least 10 seconds of VGRF data was recorded for each treatment condition.
We determined the effects of gait surface speed and grade on three response measures. We contrasted fore and hind limb *average VGRFs, peak hind limb VGRFs, and single hind limb stance duration* during the gait cycle. These values were selected to describe various aspects of the curve that may be affected by surgery or gait surface changes. All animals were trained on an instrumented treadmill (Kistler Gaitway Instrumented Treadmill, Amherst, NY) for 10 min/day for at least 5 days before surgery. We recorded both fore limb and hind limb VGRFs, as well as the center of pressure throughout the gait cycle on the front and rear force plates. VGRFs, normalized to the animal’s body weight, were compared across the four treatment conditions (two speeds and two grades) during the final day of each animal’s pre-surgery training period.

Following surgery, two motion sensors and one force sensor were used to record knee kinematics and ACL kinetics, respectively. One electromagnetic motion tracker each (Polhemus Liberty; Colchester, VT) was attached to the femur and tibia near the stifle joint line, and a force sensor (AIFP; Microstrain; Burlington, VT) was implanted into the ACL. The 3-D motions and ligament sensor response of each sheep were measured for all four treatment conditions at 2, 6, 7, 8 and 9 days after sensor implantation to determine how the animal would recover from surgery and acclimate to the transducers.

Activity order was randomized before and after surgery to avoid bias due to fatigue while monitoring VGRFs. VGRF and center of pressure data recorded after surgery were compared both to those before surgery in the same limb and to those in the contralateral limb after surgery to establish how sensor implantation affects VGRFs for each of the four treatment conditions.

This design permitted us to study two research questions: 1) *How does implanting two motion sensors and an ACL force sensor alter VGRFs and hind limb stance durations?* 2) *How does altering the gait surface speed and grade before and after surgery affect VGRFs?*
**Detailed Methods.** The study was performed in accordance with IACUC standards at the University of Cincinnati. We used an instrumented treadmill to control gait speed and grade. The treadmill includes front and rear force plates located beneath the tread to record fore and hind limb VGRFs during locomotion, respectively. The treadmill allows for VGRFs and centers of pressure to be recorded at 100 Hz for controlled speeds and grades. A handler (D Bailey) walked the animal on the treadmill using a harness while a spotter (S Herfat) ensured that the forelimbs contacted only the front force plate, while the hind limbs contacted only the rear force plate.

**VGRF Analysis of Locomotion.** A minimum of 5 consecutive and “distinct” gait cycles for each treatment combination were averaged and analyzed. A “distinct” gait cycle was defined as a cycle with clear individual left and right hoof strikes as indicated by the center of pressure recordings on the rear force plate. The center of pressure output from the force plate was used to determine differences in VGRFs between the left and right limbs. Center of pressure recordings also permitted us to establish single limb stance duration among treatment combinations. The extracted gait cycles were analyzed using a custom MATLAB program to average the gait cycles and normalize the average gait cycle time so that 0 and 1 represented consecutive left hind limb hoof strikes. Since VGRFs can vary across animals due to different weights, VGRFs were normalized to body weight. Typical curves are shown in Fig. 13 relating normalized VGRFs to normalized gait cycle for walking at 1m/s on a level surface. The peak hind limb VGRFs and the single hind limb stance durations are also indicated.

**Surgery to Implant Electromagnetic Motion Trackers and ACL Force Sensor.** The sheep was anesthetized, maintained and surgically prepped following all IACUC approved procedures and guidelines. A rumen tube was inserted to prevent bloating. The animal received IV Lactated Ringer’s
solution, and body temperature was maintained using a heated surgical table. A skin incision was created along the medial side of the patellar tendon. Once the patellar tendon was exposed, an incision was created along the medial border of the patellar tendon from origin to insertion. Care was taken to minimize the damage to the surrounding musculature and muscular attachments. The patella was then subluxed and the underlying fat pad was removed to expose the ACL. A pocket was created with a cut (< 1 cm) parallel to the long axis of the ACL in the distal third of the ACL. The AIFP was inserted so that the long axis of the sensor was perpendicular to the long axis of the ACL, and the pocket opening was sutured closed to secure the AIFP (Fig. 14). Electromagnetic tracking sensors were attached just

Figure 13  Fore and hind limb VGRFs normalized to body weight plotted against normalized gait cycle (N=10). 0 and 1 on the x-axis correspond to consecutive hoof strikes. The grey shaded regions correspond to the segment of the gait cycle when all limbs are in contact with the force plates. The letters at the top of the non-shaded regions correspond to left and right fore limb(s) (F) and hind limb(s) (H) that are in contact with the force plates.
proximal to the joint line on the medial femoral condyle and just distal to the joint line on the medial aspect of the tibia (Fig. 14). Each tracker was positioned to minimize the amount of soft tissue injury induced by tracker attachment. Wires from the AIFP and the motion tracking sensors were passed to the gluteal muscle and then cranially to the shoulder. Postoperatively, limbs were not immobilized, and animals were allowed free motion.

**Statistical Analysis.** The VGRF force data was normal and homoscedastic before and after surgery. The coefficients of variation for the pre and post op VGRFs were calculated across the gait cycle for all four combinations of speed and grade to understand inter-animal variability before and after surgery. A two-way repeated measures test was then performed on the full gait cycle to determine the effect of speed and grade, separately. Additional two-way ANOVAs were conducted for each response measure (average VGRFs, peak hind limb VGRFs and single hind limb stance duration) to determine the effects of speed and grade on these measures. For each of the four treatment levels, gait cycles for the pre vs. post op conditions were compared using a repeated measures one-way ANOVA. The three response measures for the pre vs. post op conditions were also compared using one-way ANOVAs. Lastly, a two-way

**Figure 14** Sensor implantation surgery
Top: AIFP implanted into a sagittal slit in the distal portion of the ACL and secured into the slit by placing a suture proximal and distal (arrows) to the leadwire (L).
Bottom: electromagnetic sensors (circled) implanted on the medial distal femur and medial proximal tibia.
ANOVA was performed on the post surgical data to determine the effect of changes in gait surface speed and grade, separately. The p-value for all analysis was set at \( p < 0.05 \).

### 4.3 Results

**Effect of Treadmill Speed & Grade on VGRFs before Surgery.** Increasing both treadmill speed (Table 3, Fig. 15) and grade (Table 4, Fig. 16) affected VGRFs, particularly peak hind limb forces. Increasing treadmill speed did not significantly alter average VGRFs but did significantly increase peak hind limb VGRFs. Increasing treadmill speed from 1.0 m/s to 1.3 m/s on a level surface significantly increased the sheep’s peak VGRFs \( (p<0.05) \) in both hind limbs by more than 6% during single hind limb stance. Increasing treadmill speed to 1.3 m/s on a level surface also increased the average coefficient of variation of VGRFs in both the fore limbs (slow: 8.3 ± 0.7%; fast: 12.8 ± 0.7%; mean ± SEM) and hind limbs (slow: 10.5 ± 0.7%; fast: 12.7 ± 1.1%). Increasing treadmill grade significantly shifted the VGRFs from the fore to hind limbs at both speeds and significantly increased hind limb peak VGRFs at the slower speed. Inclining the surface to 6° at 1.0 m/s decreased average VGRFs in the fore limbs by 4.4% of body weight while increasing average VGRFs in the hind limbs by 5.3% \( (p<0.05) \). Changes were similar at 1.3 m/s. Inclining the surface to 6° at 1.3 m/s produced similar decreases in fore limb average VGRFs (5.1%) and nearly identical increases in hind limb average VGRFs compared to the slower speed (5.3%). These inclination-based changes at the higher speed were also significant \( (p<0.05) \). At each speed, gait surface inclination resulted in a more even weight distribution between fore and hind limbs.

### Table 3  Effects of speed (mean ± SEM) on response measures for a level surface (pre-surgery)

<table>
<thead>
<tr>
<th></th>
<th>N = 10</th>
<th>Average VGRF (% BW)</th>
<th>Hind Limb Peak VGRF (% BW)</th>
<th>Hind Limb Contact Time (% Gait Cycle)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fore Limbs</td>
<td>Hind Limbs</td>
<td>Left*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>54.1 ± 0.8</td>
<td>45.0 ± 1.1</td>
<td>45.8 ± 1.4</td>
</tr>
<tr>
<td>1.0 m/s</td>
<td></td>
<td></td>
<td></td>
<td>28.6 ± 1.2</td>
</tr>
<tr>
<td>1.3 m/s</td>
<td></td>
<td>53.0 ± 1.0</td>
<td>45.0 ± 1.4</td>
<td>52.3 ± 2.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>32.1 ± 1.2</td>
</tr>
</tbody>
</table>

*Denotes a significant difference \( (p<0.05) \) between 1.0 m/s and 1.3 m/s
Table 4  Effects of inclination (mean ± SEM) on response measures at 1.0 m/s (pre-surgery)

<table>
<thead>
<tr>
<th></th>
<th>N = 10</th>
<th>Average VGRF (% BW)</th>
<th>Hind Limb Peak VGRF (% BW)</th>
<th>Hind Limb Contact Time (% Gait Cycle)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fore Limbs*</td>
<td>Hind Limbs*</td>
<td>Left*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>54.1 ± 0.8</td>
<td>45.0 ± 1.1</td>
<td>45.8 ± 1.4</td>
</tr>
<tr>
<td>Level</td>
<td></td>
<td>50.3 ± 1.3</td>
<td>50.4 ± 1.6</td>
<td>50.9 ± 1.6</td>
</tr>
<tr>
<td>Incline</td>
<td>49.7 ± 0.9</td>
<td>45.8 ± 1.4</td>
<td>50.9 ± 1.6</td>
<td>28.6 ± 1.2</td>
</tr>
</tbody>
</table>

*Denotes a significant difference (p<0.05) between level and incline

Figure 15  Speed did not significantly affect average VGRF (N=10) but did significantly alter hind limb peak VGRFs and hind limb peak timing. Average VGRF at 1.0 and 1.3 m/s on a level surface. 0 and 1 on the x-axis correspond to consecutive hoof strikes.

Figure 16  Grade did significantly affect average and hind limb peak VGRF at the slower speed (N=10). Average VGRF at 1.0 m/s on a level and inclined (6°) surface. Grade shifted VGRFs from the fore to the hind limbs. 0 and 1 on the x-axis correspond to consecutive hoof strikes.
Inclining the surface to 6° at the slower speed also significantly increased left and right hind limb peak VGRFs (p<0.05) by 4.6 - 4.9%. The coefficient of variation of VGRFs across the gait cycle at 1.0 m/s on an incline averaged 8.0 ± 0.5% and 9.8 ± 0.3% for the fore and hind limbs, respectively. The coefficient of variation of VGRFs across the gait cycle at 1.3 m/s on an incline averaged 15.7 ± 1.2% and 10.9 ± 0.9% for the fore and hind limbs, respectively. Across all treatment conditions, fore and hind limb VGRFs were consistent across subjects (Fig. 17) with small interanimal variability (coefficient of variation <16%).

![Figure 17](image)

**Figure 17** Fore and hind limb VGRFs were consistent across subjects (N=10) with small interanimal variability. Average VGRF at 1.0 m/s on a level surface. 0 and 1 on the x-axis correspond to consecutive hoof strikes. The dashed lines correspond to VGRF maximums and minimums.

**Effect of Surgery.** Implanting the motion sensors and force transducer did not significantly affect VGRF patterns among the four speed and inclination conditions (p>0.108). However, after extracting the three response measures (average VGRFs, peak hind limb VGRFs and single hind limb stance duration), surgery did not change average VGRFs for any condition but did significantly alter peak hind limb VGRFs and/or single hind limb stance durations for each condition.

Surgery did not significantly change average VGRFs in the fore limbs (1.6 ± 2.7%) or hind limbs (-1.6 ± 3.1%) during level treadmill walking at 1.0 m/s (Table 5 & Fig. 18). Surgery also did not significantly change peak VGRFs in the operated hind limb at the slower speed (-3.0 ± 5.2%; p=0.457).
However, surgery did significantly increase peak VGRFs in the unoperated, contralateral right limb (22.3 ± 7.0%; p=0.004). Surgery also significantly increased single limb stance duration in the unoperated right hind limb (37.6 ± 13.6%; p=0.005) but had no effect in the operated limb (-2.8 ± 3.3%; p=0.525).

Surgery did not significantly change average VGRFs in the fore limbs (3.5 ± 3.7%) or hind limbs (-3.5 ± 4.3%) during level treadmill walking at 1.3 m/s (Table 5 & Fig. 19). Surgery significantly increased the peak VGRFs for the unoperated right hind limb (25.3 ± 10.8%; p=0.036), but had no effect in the operated hind limb (-5.7 ± 12.9%; p=0.168). Surgery did not affect the single limb stance duration in the operated (0.2 ± 11.6%) or contralateral hind limb (20.2 ± 9.2%) during level treadmill walking at the faster speed (p>0.138).

Surgery did not significantly change average VGRFs in the fore limbs (7.2 ± 2.7%) or hind limbs (-6.4 ± 2.6%) during inclined treadmill walking at 1.0 m/s (Table 5 & Fig. 20). Implantation surgery did not significantly affect peak VGRFs in the left (-10.0 ± 3.9%) and right hind limbs (15.3 ± 6.0%) during inclined treadmill walking at the slower speed (p>0.072). Implantation surgery significantly increased the single limb stance duration in the unoperated right hind limb (35.1 ± 15.0%; p=0.029), but had no effect in the operated limb (-7.6 ± 8.5%; p=0.525).

Surgery did not significantly change average VGRFs in the fore limbs (9.5 ± 3.7%) or hind limbs (-8.1 ± 2.5%) during inclined treadmill walking at 1.3 m/s (Table 5 & Fig. 21). Surgery significantly decreased the peak VGRFs for the operated left hind limb (-16.7 ± 5.5%; p=0.012), but had no effect in the unoperated, contralateral hind limb (16.7 ± 7.2%; p=0.198). Surgery did not affect the single limb stance duration in the operated (-7.9 ± 3.9%) or contralateral hind limb (17.8 ± 10.7%) during inclined treadmill walking at the faster speed (p>0.082).
### Table 5: Effects of surgery (mean ± SEM) on response measures at 1.0 m/s (pre-surgery)

<table>
<thead>
<tr>
<th></th>
<th>Average VGRF (% BW)</th>
<th>Hind Limb Peak VGRF (% BW)</th>
<th>Hind Limb Contact Time (% Gait Cycle)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fore Limbs</td>
<td>Hind Limbs</td>
<td>Left</td>
</tr>
<tr>
<td>1.0 m/s Level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=6)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>53.9 ± 1.1</td>
<td>46.1 ± 1.1</td>
<td>46.3 ± 1.6</td>
</tr>
<tr>
<td>Post</td>
<td>54.7 ± 1.4</td>
<td>45.3 ± 1.4</td>
<td>44.6 ± 1.5</td>
</tr>
<tr>
<td>1.3 m/s Level</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>53.2 ± 1.5</td>
<td>46.8 ± 1.5</td>
<td>50.1 ± 3.7</td>
</tr>
<tr>
<td>Post</td>
<td>54.9 ± 1.9</td>
<td>45.1 ± 1.9</td>
<td>45.4 ± 2.1</td>
</tr>
<tr>
<td>1.0 m/s Incline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>48.9 ± 1.4</td>
<td>51.1 ± 1.4</td>
<td>53.4 ± 3.1</td>
</tr>
<tr>
<td>Post</td>
<td>52.5 ± 1.2</td>
<td>47.5 ± 1.2</td>
<td>45.9 ± 1.7</td>
</tr>
<tr>
<td>1.3 m/s Incline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(N=5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre</td>
<td>48.2 ± 2.2</td>
<td>51.8 ± 2.2</td>
<td>55.3 ± 3.2</td>
</tr>
<tr>
<td>Post</td>
<td>52.5 ± 1.4</td>
<td>47.5 ± 1.4</td>
<td>45.5 ± 1.7</td>
</tr>
</tbody>
</table>

*Denotes a significant difference (p<0.05) between pre and post surgery

---

**Figure 18** Implantation surgery did not significantly affect average VGRFs and only significantly increased the peak VGRF for the unoperated hind limb (N=6). The difference between average VGRFs before and after surgery 1.0 m/s on a level surface.

**Figure 19** Implantation surgery did not significantly affect average VGRFs and only significantly increased the peak VGRF for the unoperated hind limb (N=5). The difference between average VGRFs before and after surgery at 1.3 m/s on a level surface.
Effect of Treadmill Speed and Inclination on VGRFs after Surgery. After surgery, increasing treadmill speed affected peak VGRFs and single limb stance duration, but increasing treadmill grade had no effect. During level treadmill walking, increasing speed significantly increased both the single limb stance duration of the operated hind limb (p=0.032) and peak VGRF for the non-operated hind limb (p=0.01). Increasing treadmill speed to 1.3 m/s on a level surface also significantly increased the average coefficient of variation of VGRFs in both the fore limbs (slow: 12.4 ± 1.4%; fast: 17.3 ± 2.7%; mean ± SEM) and hind limbs (slow: 12.9 ± 1.7%; fast: 16.4 ± 3.2%). During inclined treadmill walking, increasing speed significantly increased the single limb stance duration of the operated hind limb (p=0.039). Altering the treadmill grade did not significantly affect any response measures. The coefficient of variation of VGRFs across the gait cycle at 1.0 m/s on an incline averaged 13.3 ± 1.7% and 11.8 ± 2.0% for the fore and hind limbs, respectively. The coefficient of variation of VGRFs across the gait cycle at 1.3 m/s on an incline averaged 14.1 ± 2.1% and 11.4 ± 2.4% for the fore and hind limbs, respectively. Across all treatment conditions after surgery, fore and hind limb VGRFs were consistent across subjects with small interanimal variability (coefficient of variation <18%).

Figure 20 Implantation surgery did not significantly affect average VGRFs or hind limb peak VGRFs (N=5). The difference between average VGRFs before and after surgery at 1.0 m/s on an inclined (6º) surface.

Figure 21 Implantation surgery did not significantly affect average VGRFs and only significantly decreased the peak VGRF for the operated hind limb (N=5). The difference between average VGRFs before and after surgery at 1.3 m/s on an inclined (6º) surface.
4.4 Discussion

Measuring vertical ground reaction forces (VGRFs) on an instrumented treadmill provides a non-invasive and repeatable method to establish normal gait patterns and to examine the effects of speed, grade and surgical treatments on gait. Our VGRF measurements acquired before and after surgery are consistent among animals, with a coefficient of variation averaging no more than 18% for all activities using an instrumented treadmill. This low inter-animal variability suggests that changes produced by different treatments will not be masked by differences among animals. The fact that increasing treadmill surface speed increased hind limb peak VGRFs but did not affect average VGRFs could be attributed to the small range in speeds that the sheep tolerated (1.0 to 1.3m/s). Since increasing grade (at 1 m/s) significantly increased both hind limb average and peak VGRFs prior to surgery, it would appear that altering grade may be a more effective way to alter hind limb loading and ACL forces in future experiments.

Sensor implantation produced only modest changes in VGRFs in the hind limbs, but the animals spent greater time in single limb stance in the non-operated limb early post surgery. While we expected decreases in both average and peak VGRFs in the operated limb, we also sought to ensure that inserting these sensors would not reduce VGRF forces to less than 80% of pre-surgery levels. The fact that peak VGRFs in the operated limb exceeded 80% of pre-surgery levels and average VGRFs in both fore and hind limbs exceeded 90% across all treatment conditions indicates that we have achieved our acceptance criterion. Achieving this threshold across all conditions also means that our strategy to measure knee kinematics and kinetics in the operated animal is a suitable model for normal gait. Sensor implantation surgery also produced differing effects in the operated vs. non-operated limbs. Although the duration of single limb stance in the operated limb never decreased by more than 8% during gait, duration of single limb stance in the non-operated limb increased by as much as 37.6 ± 13.6 % (during level walking at 1.3 m/s). While we expected this initial shift from the operated to non-operated limb
early post surgery, we would have preferred to allow each animal an even longer recovery period to bring the VGRFs and durations closer to pre-surgery levels, but the reliability of the sensor recordings decreased after the first 8 days post surgery.

Our VGRFs for an ovine walking on an instrumented treadmill are similar to previous findings in the literature with even lower interanimal variability. Our hind limb average VGRFs on a level surface are within the range reported by Tapper et al. [146] (34.5–50.0% BW) for ovine overground walking (1.0 – 1.6 m/s). Our peak hind limb VGRFs are also similar to average peak pre-operative hind limb VGRFs of 48% BW reported by Taylor et al. [250] for ovine overground walking (average speed of 0.97 m/s). The inter-animal variability in our study is also lower than the 30% variability reported by Duda et al. [157] who recorded average hind limb VGRFs of ≈ 60% BW when walking sheep on a walkway at a slower uncontrolled speed (≈ 0.7 m/s). We attribute this lower variability to the instrumented treadmill that we used to precisely control gait surface speed. Controlling such gait parameters is critical as we and others seek to determine and universally apply functional tissue engineering parameters as design criteria and evaluation benchmarks in the ovine and other model systems.

The varying effects of speed and grade on VGRFs in the ovine model have not been measured previously in the ovine model and will be important when designing future studies. Before surgery, increasing speed did not have a substantial effect on gait but did significantly increase the average coefficient of variation, while increasing treadmill grade did have a substantial effect on gait and did not increase the average coefficient of variation. Based on these pre-op findings, adjusting treadmill grade at the slower speed will allow us to consistently and significantly alter the loading on the hind limbs for future experiments. Our post-op findings also support this strategy as increasing treadmill speed also had minimal effects on response measures after surgery. Because increasing treadmill grade did significantly affect response measures before surgery but not after surgery, we believe that early after surgery the sheep may tend to shift its center of gravity in an anterior direction to protect the operated
hind limb, thereby lowering VGRFs in the operated limb. Since each animal’s response to surgery is variable, we will test more animals to determine if there is a true effect of grade after surgery. We will also attempt to expand the range of gait surface grade for each animal to ensure significantly different activities of daily living based on VGRFs. While our finding that increasing gait surface speed increases peak VGRFs supports similar results for humans [251-255] and canines [256], the effect of increasing speed on average VGRFs has not been reported in any species. Also, the effect of increasing treadmill grade on VGRF magnitudes during walking has only been reported in the human, for which findings are few and inconsistent. For humans, peak VGRFs are conventionally reported for the early stance peak and late stance peak due to the bimodal VGRF pattern. Increasing inclination resulted in increased peak VGRFs during overground locomotion for the early stance peak [257] and the late stance peak [258]. While our findings in the sheep hind limbs are similar in that we also found that increasing treadmill grade increases peak VGRFs, caution should be exercised when comparing results for a biped and quadruped.

Our study is not without limitations. Our finding that average VGRFs were not affected by changing speed suggests that we did not impose a broad enough range of treadmill speeds. Although we attempted a wider range of speeds, the sheep did not exhibit normal gait at speeds less than 1.0 m/s and could not maintain traction on the treadmill at speeds greater than 1.3 m/s after surgery. Based on the lack of speed-related effects on VGRFs, future experiments will hold the treadmill speed at 1.0 m/s. Similarly, our results only reflect changes over a relatively small range of inclinations. Treadmill grades greater than 6° were also attempted, but the animals were unable to maintain traction on the instrumented treadmill. To increase the range of treadmill grades and VGRFs, we are now studying the effects of downhill treadmill position. While this position decreases hind limb loading, the altered joint motions and increased knee extensor muscle forces should provide a broader range of ACL loading patterns to examine. Another limitation is that even by using individual front and rear force plates, we
still cannot determine VGRF contributions of each limb throughout the entire gait cycle. For example, both hind limbs are in contact with the rear force plate for a portion of the gait cycle, preventing measurements of individual limb patterns. We could therefore analyze hind limb peak VGRFs and stance durations only when a single limb was in contact. Any minor weight redistributions made by the animal during double leg stance are thus undetectable in this system. Also, we could not rely on VGRF and center of pressure data if a hind limb contacted the front force plate or vice versa. It was important to center the animal between the two force plates throughout the gait cycle. We used a harness to keep the animal centered on the treadmill as well as a spotter who ensured proper contact of the limbs with the force plates. However, we could not be certain of the effect of the harness on our measurements.

Future experiments will examine how additional activities as well as isolated and combined knee ligament injuries affect VGRFs, knee motions and ACL forces. An instrumented treadmill provides a repeatable setting to more efficiently measure VGRFs during normal locomotion and following surgical treatment. Using this methodology, we are now correlating VGRFs with 3-D knee motions and ACL forces during realistic activities of daily living. Such measurements complement our prior study showing correlations between VGRF and in vivo ACL force in the goat model, most importantly that ACL forces increase with increasing VGRF [203]. These correlations will potentially reduce the implanted instrumentation currently needed to obtain FTEPs, as well as evaluate normal function and repair outcome in vivo. These results should permit us to establish design criteria and evaluation benchmarks for traditional ACL reconstructions as well as more novel tissue engineered treatments. Ultimately, these FTEPs will guide research by our group and others as we seek to better understand in vivo joint function during activities of daily living in large animal models and eventually in patients.
The overall objective of the *in vivo* studies is to simultaneously record vertical ground reaction forces (VGRFs), 6 degree of freedom (DOF) joint kinematics, and the output of the arthroscopically implantable force probe (AIFP) in the ACL. As described in the previous chapter, VGRFs were successfully recorded before and after surgery to determine the effect of implantation surgery on the gait of the animal. We also simultaneously recorded the 6 DOF kinematics of the stifle joint and the voltage output of the arthroscopically implantable force probe (AIFP) implanted in the ACL.

While the VGRFs were relatively easy to record, recording AIFP output and reliable knee motions presented a greater challenge. This section will discuss our attempts at reproducing the subject-specific *in vivo* joint motions using a 6 DOF robot and a method to improve our *in vivo* motion recording accuracy. Our goal is to use the AIFP output to validate the reproduced joint motions. While the AIFPs frequently failed prior to the final day of *in vivo* testing, we were able to successfully record AIFP output for a few days after surgery. In addition, replacing the damaged AIFPs *in vitro* reproduced the AIFP voltage patterns, allowing for the *in vitro* validation of the *in vivo* AIFP voltage recordings.

### 5.1 Measurement of *In Vivo* AIFP Output

Eight animals were used to examine AIFP voltage output based on activity level. AIFP voltage outputs were recorded for the four activity levels (1.0 m/s – level, 1.0 m/s – incline, 1.3 m/s – level, 1.3 m/s – incline) at 2, 5, 6, 7, 8, and 9 days after surgery.

**Results.** All AIFP sensors were functional during the first day of post op testing. However, six of the eight AIFPs failed prior to the final day of testing. While the AIFPs were functional, AIFP transducer
output was greatest when the operated (left) hind limb contacted treadmill surface (Fig. 22) and decreased prior to the right hind limb lifting off. Through most of single hind limb stance, AIFP output remained fairly constant. AIFP output then decreased as the right hind limb contacted the treadmill surface and reached its baseline value as the operated limb lifted off. Although increasing treadmill speed (Fig. 22) did slightly increase the baseline sensor level and increasing treadmill grade slightly decreased the baseline sensor level, neither significantly changed maximum sensor output. Changes in speed and inclination also had little effect on the shape of the AIFP voltage curve during the gait cycle.

**Challenges & Proposed Solutions.** In spite of the high incidence of AIFP failure, we will continue implanting the AIFP sensor at surgery. Only motions that are independently validated by the AIFP output will be used to measure joint kinetics. A valid motion will require that the AIFP output pattern for the simulated *in vivo* motion matches the *in vivo* AIFP output. This will be difficult to achieve if the AIFPs continue to fail *in vivo*. Thus, we have developed a solution to increase the durability of the AIFPs. The majority of the AIFPs failed at the lead wire connections on the sensor. To address this issue, we have begun to apply a protective polymer coating to this connection, making sure not to affect the elastic deformation of the sensor, which would alter its sensing capabilities. This protective coating
increased the durability of the AIFP during in vitro experiments which required the insertion, removal, and reinsertion of the AIFP in the ACL (section 5.2).

We must also address the lack of change in AIFP output between activities. The goal is to measure in vivo parameters for various activities that challenge the ACL differently. The range of gait surface grades may not have been large enough to significantly alter ACL force. Therefore, we will introduce a downhill activity to broaden our range of grades in an effort to use gait surface grade to produce a significant change in AIFP output. Our inability to determine changes in AIFP voltage output for increasing gait surface grade could have been due to the AIFP measurements being influenced only by the fibers in contact with the body of the AIFP. Therefore, the ACL fibers that were affected by the gait surface inclination may not have been measured. If the increased range of grades does not cause a significant change in AIFP output, we will investigate implanting the AIFP in a different location in the ACL or alter its orientation within the ACL.

5.2 Effect of AIFP Replacement on AIFP Output

Two normal ovine hind limbs (not pairs) were used to investigate the effect of replacing the AIFP in vitro. Each joint was prepared and setup in the robot using the same procedure described in Chapter 3. A sagittal incision (length < 1 cm) was created in the distal portion of the ACL. Using the same AIFP implantation method which was used in vivo (Chapter 4), the AIFP was inserted into the incision, oriented with the gap in the sensor pointed distally, and secured by placing sutures proximal and distal to the leadwire (Fig. 23).  

Figure 23 AIFP (right) implanted into a sagittal incision of a soft tissue structure (left). Each “S” indicates a suture location. (MicroStrain AIFP Manual)
Once the AIFP was implanted in the ACL, we applied 10 cycles of the simulated ovine in vivo motion path adapted from Tapper et al. [152] to measure the AIFP output. The sutures and AIFP were then removed. The AIFP was reinserted in the same location, only ensuring that the gap in the sensor was pointing distally, and secured again using sutures. Another 10 cycles of the ovine gait path were applied again to measure AIFP output. Next, the remaining soft tissue (excluding the ACL) and the distal portions of the femoral condyles were removed to ensure that the ACL was the only structure transmitting force across the joint. We applied another 10 cycles of the ovine gait path to measure the force in the ACL using the robot’s load cell.

**Results.** Reinserting an AIFP into the same incision did not significantly affect the magnitude or shape of the AIFP voltage curve (Fig. 24). The average magnitude change was 14.4 ± 11 (SD)%.

**Discussion.** We concluded that an AIFP can be replaced in vitro following sensor failure without significantly changing the AIFP-ACL interaction. While we initially planned to use the AIFP to measure actual ACL force, we will now instead use the AIFP to independently verify the accuracy of the reproduced motions. ACL force will be measured using the 6-axis load cell attached to the robot using the procedure previously presented in Chapters 2 and 3.

5.3 Accuracy of Electromagnetic Tracking System for Knee Motion Measurement

To determine the accuracy of our motion measurement system, we analyzed the measurement error for translations and rotations using our robot. Motions were recorded using an electromagnetic tracking sensor (Liberty; Polhemus Corp., Burlington, VT) attached to a polymeric fixture on the robot.
end effector. The sensor coordinate axes were aligned with the robot axes. Translations and rotations were applied individually along and about each axis, respectively. Translations also were imposed as a sine wave with a mean level of 0 mm, amplitude of 25 mm, and a 3 second period. Rotations were imposed in the form of a sine wave with a mean level of 0°, amplitude of 30°, and a 3 second period. The corresponding outputs from the electromagnetic system and robot were compared and percentage errors were computed for all translations and rotations.

**Results.** Percent errors for the translations averaged $5.6 \pm 2.1$(SEM)%, $5.2 \pm 3.5\%$, and $3.1 \pm 2.4\%$ along the x, y, and z axes, respectively. Percent errors for the rotations averaged $1.8 \pm 2.5\%$, $1.2 \pm 2.2\%$, and $2.2 \pm 1.4\%$ about the x, y, and z axes, respectively. Based on the ranges of motion exhibited by the ovine stifle joint during a walking activity [146], the error of our *in vivo* joint kinematics measurements could reach 0.17 mm for translations and 0.37° for rotations.

**Discussion.** The errors associated with the electromagnetic trackers are less than the errors reported for an instrumented spatial linkage (0.5 mm and 0.5°) [259] and optical markers [260, 261], but larger than those for biplane fluoroscopy [238]. However, due to the high cost of building and maintaining a biplane fluoroscopy unit and the relatively small decrease in error, the electromagnetic tracking system is an acceptable technology for our application. However, investigators must limit the amount of metal in the field when using the electromagnetic system as metal can cause a substantial increase in measurement error. Our measurements were taken in the robot environment rather than in the actual *in vivo* measurement recording environment. Although our *in vivo* recording environment does contain a large treadmill constructed with metal components, the robot environment contains more metal in the field at a closer proximity to the EM sensors and source. Thus, these measurements should provide a worst-case scenario for determining the EM measurement error.
5.4 In Vivo Knee Motion Measurements Using the Electromagnetic Tracking System

Surgery was performed in seven animals to implant an AIFP in the ACL and two electromagnetic (EM) trackers on the medial distal femur and the medial proximal tibia. The EM trackers allowed us to measure the 6 DOF motions of the tibia and femur. Of the seven animals, four animals provided usable kinematic data. The motion data for the three removed animals was lost for various reasons. Data files for one animal were corrupt, one EM tracker wire was chewed through by another animal, and one EM tracker was detached from its wire. After the final day of *in vivo* testing, each ovine stifle joint was dissected down to the joint capsule, leaving the major ligaments and menisci intact. Each joint was rigidly mounted to our robot fixture and a 3D coordinate measurement machine (CMM, Model #F04L2; FARO Technologies Inc., Lake Mary, FL) was used to acquire the 3D coordinates of the EM tracker surfaces and the anatomical points on the joint needed to establish the tibial and femoral joint coordinate systems. These measurements were then input into a custom MATLAB program that transformed the EM tracker measurements into anatomical motions based on the knee joint coordinate system [198].

**Results.** The average 6 DOF ovine stifle kinematics from the four subjects are presented in Fig. 25. The motions represent the translations and rotations of the tibia with respect to a stationary femur.

**Discussion.** Our joint kinematics measurements are similar in shape to those recorded by Tapper et al. [146], but our flexion-extension range is approximately 15° less with the joint being more flexed at hoof-strike. Our kinematic measurement system differed (electromagnetic vs. optical) which could have produced different measurements and measurement errors. Also, either method could have altered gait if motion measurement hardware was placed in a position that obstructed the natural movement and function of the muscles surrounding the knee. In our study, vertical ground reaction forces (VGRFs) were monitored before and after surgery to detect any changes in gait. Our results indicated that following surgery, the animals into which we implanted motion tracking and force sensors
maintained a minimum of 80% of pre-surgery VGRF values. However, we have encountered other evidence that our motion measurement errors may be higher than expected. The other observations which support this conclusion are discussed in the following sections.

![Sheep Knee Kinematics](image)

**Figure 25** The 6 DOF ovine stifle joint kinematics (Mean ± SEM). The translations are solid lines, and the rotations are the dashed lines. The colors correspond to motions about the same axis.

### 5.5 Applying Subject-Specific Simulated Motions to Measure Intact Knee and ACL Force

The four ovine knees for which *in vivo* knee kinematics were successfully recorded were prepared and setup in the robot using the same procedure described in Chapter 3. The electromagnetic trackers were not removed from the tibia and femur so that we could determine the tracker positions with respect to the robot coordinate system. Once the tibia and femur were secured to the robot fixtures, we used the CMM to digitize 1) the anatomical coordinates needed to establish the tibial and
femoral coordinate systems, and 2) the surfaces of the tibial and femoral trackers. These measurements were input into a custom MATLAB program that calculated 1) the electrical center of each tracker with respect to the joint coordinate system [198], 2) the position of the tibial tracker with respect to the femoral tracker, and 3) the robot move required to achieve a chosen starting point from the in vivo electromagnetic tracker data. The electrical center of the tibial tracker was then set as the robot “tool”, which means that all translations and rotations were performed about this point. The robot was then adjusted accordingly to reach the starting point. Following the move, we again digitized the sensor surfaces and anatomic points to determine the actual position of the tibial tracker with respect to the femoral tracker. We aimed to achieve a starting point in our motion to within 0.1 mm for the translational coordinates and within 0.1° for the rotational coordinates.

Challenges. The EM motion measurement errors became evident when attempting to reproduce the recorded EM motions in the robot and apply them to the same operated joint. For two of the limbs, we could not achieve the starting point due to large motion measurement errors. For the other two joints, we were able to set the starting position with respect to 5 of the 6 degrees of freedom (DOF); however, one DOF (medial-lateral displacement) was always unachievable without damaging the joint.

Figure 26 Intact knee and ACL forces for a simulated in vivo motion (N=2). Anterior-posterior force (top) and compression-distraction force (bottom) for a normalized gait cycle starting and ending at consecutive hoof-strikes.
Therefore, we identified the need to develop a strategy to reduce our *in vivo* motion measurement error. To increase our motion measurement accuracy, a calibration factor will be computed for each joint and later applied to the kinematic data to reduce the effects of EM tracking error (section 5.6).

**Force Measurement.** The intact knee and ACL forces were measured for the two joints for which we achieved a starting point with respect to 5 of the 6 DOF. Once the starting position was achieved, we applied 10 cycles of the subject-specific walking motion. The average intact knee and ACL force for these two knees are presented in Fig. 26. During stance, peak ACL force occurs near midstance. However, the ACL remained minimally loaded throughout stance. The ACL force peaks during swing phase and contributes over half of the total force exhibited by the joint at peak flexion.

**5.6 Proposed Solutions for Future Studies**

In our subsequent experiments we will make several changes to ensure accurate AIFP voltage and knee motion measurements. To better protect the leadwires, we have redesigned the vest, which is applied to the sheep post surgery, to prevent access to the leadwires from the subject and other animals. To increase the durability of the AIFP, we will apply a polymer coating on the junction of the lead wires and the sensing unit of the AIFP. The previous study revealed the need to improve our *in vivo* motion measurement accuracy. First, we will keep the electromagnetic (EM) source in the same position and orientation for all *in vivo* measurements. Maintaining a consistent source position and orientation should produce more consistent kinematic data between trials with the same degree of measurements errors in each degree of freedom. To further improve on our *in vivo* motion measurement accuracy, we will also determine the measurement error for the Polhemus EM system in the *in vivo* environment by digitizing the location of the two electromagnetic trackers using an external validation method. The CMM will be used to validate the EM tracker measurements at different joint positions. Following the final day of *in vivo* data acquisition, we will harvest the operated limb and
expose the EM sensors. The femur will be rigidly attached to a custom-made fixture that will sit atop the treadmill to simulate joint position. The joint will be passively extended to its minimum knee angle and the tibia will be rigidly secured. Once the joint is rigidly fixed in this position, EM tracker positions will be recorded using the EM system and the surfaces of the EM sensors will be digitized using the 3D measurement device. The joint will then be flexed by four even increments to achieve the full in vivo range of flexion, and the EM and 3D measurement device measurements will be acquired at each position. The surface planes determined from the 3D measurement device will be used to calculate the electrical center of each of the EM sensors. The distance and orientation of the tibial tracker with respect to the femoral tracker will be computed using the EM system measurements and computed again using the CMM measurements. The EM results will then be plotted versus those from the CMM to develop a calibration factor for the in vivo kinematic measurements acquired by the EM system. Thus, the CMM will serve as an external validation tool. The calibration factor for each subject will be applied to the in vivo motion measurements for the same subject to produce a more accurate motion path.

5.7 Summary of In Vivo Experiments

These studies have helped to develop the methodology which we plan to use to determine the in vivo function of the ovine stifle joint and its structures during activities of daily living. We are now closer to using our novel methodology to define functional tissue engineering parameters (FTEPs) in a large animal model where surgeries can be performed in a more reproducible manner. Such a model offers the opportunity to more rapidly evaluate both traditional and novel tissue engineered ACL reconstructions. The instrumented treadmill has been critical in this process by helping us to establish gait patterns in the normal sheep for controlled activities and to discover how surgery alters these patterns. Introducing electromagnetic trackers into this research has provided a method for measuring in vivo knee kinematics.
In vivo force measurement has been our greatest challenge to date. We must continue to improve the durability of these sensors in order to successfully record AIFP output out to 9 days after surgery. However, if we continue to experience premature AIFP failures in the proposed studies, we are confident that replacing the AIFP in vitro will not affect ACL-AIFP interaction and thus will not change ACL voltage output patterns. Access to a 6 DOF robot has provided many research opportunities in the area of orthopaedic biomechanics. The system allows us to apply physiologic 6 DOF motions to determine the corresponding joint and tissue forces (Chapters 2 & 3). Combining the technologies used to measure in vivo parameters (e.g. the instrumented treadmill, EM tracking system, and AIFP) with the capabilities of a 6 DOF robot will allow us to: 1) advance our understanding of normal knee and ACL function; 2) develop correlations between joint and ACL kinetics, knee kinematics, and VGRFs; 3) investigate the effects of various injuries and treatments on knee and tissue function; 4) establish functional tissue engineering parameters which will serve as design criteria and evaluation benchmarks for orthopaedic treatments; and 5) evaluate orthopaedic treatments.
Chapter 6

Effect of Perturbing a Simulated In Vivo Motion on Knee and ACL Kinetics [262]

Current surgical treatments for common knee injuries do not restore the normal biomechanics, increasing the susceptibility to the early onset of osteoarthritis. In pursuit of improving long term outcome, investigators must understand normal knee kinematics and corresponding joint and ACL kinetics during activities of daily living. Our goal is to measure in vivo joint motions for the ovine stifle model and later simulate these motions with a 6 degree of freedom (DOF) robot to measure the corresponding 3D kinetics of the knee joint and ACL. Both motion measurement and motion simulation technologies have associated errors.

The objective of this study was to determine how motion measurement and motion recreation error affect knee joint and ACL kinetics by perturbing a simulated in vivo motion in each DOF and measuring the corresponding joint and ACL forces and moments. The ACL forces measured across all perturbations were compared to the ACL force-displacement curve of each ACL to determine the portion of the curve in which the ACL is functioning. The average, resultant ACL force for all perturbations was less than 10% of the ACL failure load.

Only translational perturbations significantly affected the intact knee and ACL kinetics. The compression-distraction perturbation had the largest effect on intact knee forces and the anterior-posterior perturbation had the largest effect on ACL forces. These translational errors should be addressed for future studies to provide a more accurate representation of the intact knee and ACL kinetics.
6.1 Introduction

Knee soft tissue injuries are very common and can cause osteoarthritis even after surgical treatment. With as many as 250,000 ACL injuries occurring annually [263], ACL tears are among the most common knee injuries [264], especially during sports activities [58, 265]. While success rates exceeding 90% have been reported for ACL reconstruction [18, 19], native knee kinematics are not restored, leading to abnormal knee joint laxity and instability [21, 22, 28]. The inability to restore normal ACL knee kinematics can increase the risk for early onset of osteoarthritis [20]. In fact, several studies have indicated no difference in the long term prevalence of knee osteoarthritis between ACL reconstructed and conservatively treated patients following ACL injury [14, 266, 267]. Therefore, more effective treatment strategies are required to restore normal knee biomechanics and thereby prevent the accelerated onset of osteoarthritis following ACL injury.

The inability of current ACL reconstructions to restore normal knee biomechanics can be attributed to the fact that normal ACL and reconstructed ACL biomechanics are traditionally determined from motions that reproduce clinical examinations (e.g. anterior drawer tests or pivot shift tests) [41, 42, 201] rather than activities of daily living (ADLs). While these previous studies have provided valuable information about the role of the ACL, key information regarding ACL biomechanics remains unavailable.

Although in vivo joint or tissue kinetics cannot be directly measured, improved measurement and motion recreation technologies now provide the capabilities to accurately record and reproduce in vivo motions in the laboratory in order to determine corresponding tissue and joint kinetics. However, an appropriate preclinical animal model is required to acquire both the in vivo and in vitro measurements. The ovine stifle joint is an attractive model for these studies because it is a suitable orthopaedic injury and surgical model for the human knee joint and its soft tissue structures [145, 155, 241]. Furthermore, full 3D knee kinematics have been measured for the ovine model [146].
Our research goal is to measure *in vivo* motions for the ovine stifle model and recreate these motions using a 6 degree of freedom (DOF) robot to measure knee joint and ACL kinetics. Unfortunately, both the measurement and motion simulation technologies have associated errors, which could significantly affect the forces and moments recorded in both the intact knee and ACL. The implication of the associated errors is that depending on the position on the force-displacement curve (toe or linear region) where the joint or ACL functions for the ADL, small positional errors could have a large effect on force or moment measurements. The objective of this study is to determine how perturbing a simulated *in vivo* motion affects knee joint and ACL 3D kinetics. We hypothesized that small translational or rotational perturbations of the motion would significantly affect the intact knee force but not the ACL force because knee structures can achieve up to 40% of failure strength for ADLs [203], while the ACL has been shown to function at less than 10% of its failure strength [158].

### 6.2 Design & Methods

*Experimental Design.* Six (3 left and 3 right; no pairs) hind limbs from skeletally-mature, mixed breed female sheep (3–4 yrs old; 50-78 kg) were used. The ovine model was chosen because the stifle joint is morphologically and biomechanically similar to the human knee [145, 155]. A Kuka robot (KR210, Kuka Robotics Corp., Clinton Township, MI) with a 6-axis load cell (Theta Model, ATI Industrial Automation, Apex, NC) was used to recreate a 6 DOF ovine *in vivo* motion adapted from Tapper et al. [146]. The intact knee joint was subjected to 10 cycles of this gait path while joint forces and moments were recorded. We determined the effects of perturbation by comparing the forces and moments recorded for the intact knee joint when the motion was applied at the normal starting position vs. a perturbed tibial starting position. For each of the 6 degrees of freedom (translations along and rotations about the X, Y, and Z axes of the tibial joint coordinate system), the intact knee joint was first exposed to 10 cycles of the ovine gait path at the normal starting position and subsequently perturbed by four levels (-0.50, -0.25, 0.25, and 0.50; mm or deg). This range of translational and rotational perturbations
were chosen to bound the errors associated with our motion measurement (translation error: 0.17 mm; rotational error: 0.37°) and recreation (translation error: 0.15 mm; rotational error: 0.15°) technologies. The distal femoral condyles and all soft tissue except for the ACL were then removed. The perturbations procedure was repeated for the ACL-only condition.

Finally, each ACL was harvested and tested in tension to determine the force-displacement curve. The ACL forces recorded for the perturbed motions were compared to the failure curve to determine the region of the curve where the ACL operates and the percentage of failure force the ACL experiences during an ADL.

**Joint Preparation and Robot Setup.** Ovine limbs were obtained from a local vendor. All limbs were prepared and tested according to our previously reported protocol [153]. Each limb was dissected free of all muscles and tendons, leaving the knee joint capsule, the collateral and cruciate ligaments, and both menisci intact. The proximal half of the tibia was secured in a specially-designed fixture using polymethyl methacrylate (PMMA). The tibial fixture was used to establish the tibial joint coordinate system (TJCS) [198]. The tibial fixture was attached to the load cell on the 6 DOF robot and adjusted to align the TJCS with the robot and load cell axes. The centroid of the tibial ACL insertion site was selected as the tibial joint center point and digitized using a 3D coordinate measurement machine (CMM, Faro Digitizer Model #F04L2; FARO Technologies Inc., Lake Mary, FL). All rotations and translations were imposed about this point, and all forces and moments were recorded about this point.

The hanging femur was then guided onto the base fixture and the forces and moments were zeroed before securing the femur. The joint was then positioned at a 60.5° flexion angle and small translational adjustments were made in the robot to minimize forces and moments to <5 N and <1 Nm, respectively. This flexion angle was selected because it corresponds to the midpoint of knee flexion for the selected motion path, where we believe that the forces and moments would be minimal.
**Robot Testing.** All testing was performed at room temperature. With the joint in the starting position, a set of 10 gait cycles (8.22 sec cycle duration) was applied to precondition the joint. We applied another 10 gait cycles and recorded the forces and moments. We then perturbed the starting position in each DOF by each level (-0.50, -0.25, 0.25, and 0.50; mm or deg). The 10 cycles were repeated following each perturbation while forces and moments were recorded. Finally, we removed the soft tissue structures and the distal portion of the femoral condyles, leaving the ACL as the only structure transmitting force across the joint. The testing procedure was repeated for the ACL-only condition.

**ACL Failure Testing.** The ACL with bone blocks at each end was harvested from each limb and failed in tension. The ACL sample was oriented to align the fibers linearly prior to acquiring gauge length, width, and thickness measurements using a digital caliper. Each bone block was potted inside a custom fixture using PMMA. The fixtures were attached to the material testing system (Instron 8501; Instron, Norwood, MA). Each sample was preconditioned for 50 cycles with a strain amplitude of 3%. The ACL was failed in tension using a strain rate of 20% per sec while acquiring the force-displacement curve. The failure force-displacement curve for each ACL was compared to the forces measured throughout the range of perturbations for the ACL condition of the same subject. Using this approach, we estimated the region on the force-displacement curve where the ACL operated during a simulated in vivo motion.

**Data Analysis (Robot Perturbation Experiments).** For the intact knee and ACL, we examined the forces along and the moments about each anatomical axis. The 8<sup>th</sup> and 9<sup>th</sup> gait cycles of the 10 cycle test were used for analysis to minimize any viscoelastic effects. The 8<sup>th</sup> and 9<sup>th</sup> gait cycles were averaged and normalized to consecutive heel strikes. The forces and moments were averaged across subjects for the normal condition and each level of perturbation. Forces were analyzed with respect to the tibial joint coordinate system (TJCS) along the anterior-posterior (A-P), medial-lateral (M-L), and compression-
distraction (C-D) axes. Moments were analyzed with respect to the TJCS according to the abduction-adduction (Ab-Ad), flexion-extension (F-E), and internal-external (I-E) anatomic rotations about the A-P, M-L, and C-D axes, respectively.

**Statistical Analysis.** For each direction of force and moment, a one-way repeated measures analysis of variance (ANOVA) was performed to determine if the perturbation produced a significant difference. All data was normal and homoscedastic. Post hoc comparisons were made using the Bonferroni method. The significance level for all comparisons was set at p < 0.05.

**6.3 Results**

**Intact Knee.** Only translational perturbations significantly affected the intact knee kinetics (Table 6). The C-D perturbation had the largest effect on the intact knee forces throughout the gait cycle (Fig. 27), with the full range of C-D perturbation (distraction to compression shift) significantly increasing (p < 0.05) the force in the compression direction throughout gait (stance: -359.4 ± 8.5 (SEM) N; swing: -220.2 ± 26.5 N). The other translational perturbations only significantly affected (p < 0.05) the intact knee forces during swing (Table 6; A-P perturbation shown in Fig. 28).

**ACL.** Only the A-P and C-D perturbations affected ACL kinetics (Table 7). The A-P perturbation had the greatest effect, particularly on ACL A-P forces (Fig. 29) and F-E moments, as the full range of perturbation (posterior to anterior shift) significantly increasing (p<0.05) the ACL A-P forces in the anterior direction (stance: 7.7 ± 1.8 N; swing: 39.7 ± 3.4 N) and F-E moments in the flexion direction (stance: 0.5 ± 0.1 Nm; swing: 2.7 ± 0.2 Nm). All significant effects of the perturbations on ACL forces and moments are shown in Table 7.

*The average ACL resultant forces across all subjects and perturbations were less than 10% of the average ACL failure load.* The average ACL gauge length, width and thickness were 19.6 ± 0.5 mm, 8.0 ± 0.8 mm and 5.75 ± 0.3 mm, respectively. The average failure force and stiffness of the ACL tested in tension were 1805.0 ± 42.9 N and 435.4 ± 16.2 N/mm, respectively (Fig. 30; N = 5). During stance phase,
the ACL resultant forces across all perturbations and subjects ranged from 0.1 to 23.2 N. During swing phase, the ACL resultant forces across all perturbations and subjects ranged from 0.2 to 168.9 N. For each subject, the ACL resultant force for each perturbation remained within the toe region of the force-displacement curve throughout the gait cycle.

<table>
<thead>
<tr>
<th>Table 6 Effect of Perturbations on Intact Knee Kinetics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perturbed Direction</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td><strong>Stance</strong></td>
</tr>
<tr>
<td>C/D</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td><strong>Swing</strong></td>
</tr>
<tr>
<td>A/P</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>M/L</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>C/D</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
### Table 7  Effect of Perturbations on ACL Kinetics

<table>
<thead>
<tr>
<th>Perturbed Direction</th>
<th>Significantly Different Conditions</th>
<th>Significance</th>
<th>Increased Force(s)/Moment(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stance</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/P</td>
<td>Neutral</td>
<td>p &lt; 0.033</td>
<td>Anterior, Medial, Distraction Forces Flexion Moment</td>
</tr>
<tr>
<td></td>
<td>0.25 mm Posterior ≠ 0.50 Anterior</td>
<td>p &lt; 0.008</td>
<td>Anterior &amp; Medial Forces Flexion &amp; Adduction Moments</td>
</tr>
<tr>
<td></td>
<td>0.50 mm Posterior ≠ 0.50 Anterior</td>
<td>p &lt; 0.019</td>
<td>Anterior, Medial, Distraction Forces Flexion &amp; Adduction Moments</td>
</tr>
<tr>
<td>A/P</td>
<td>0.25 mm Posterior ≠ 0.25 mm Anterior</td>
<td>p &lt; 0.021</td>
<td>Anterior Force Flexion Moment</td>
</tr>
<tr>
<td></td>
<td>0.50 mm Posterior ≠ 0.25 mm Anterior</td>
<td>p &lt; 0.015</td>
<td>Anterior &amp; Medial Forces Flexion &amp; Adduction Moments</td>
</tr>
<tr>
<td><strong>Swing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A/P</td>
<td>0.50 mm Distraction ≠ 0.50 mm Compression</td>
<td>p &lt; 0.032</td>
<td>Posterior Force Extension Moment</td>
</tr>
</tbody>
</table>
Figure 27  C-D perturbations significantly affected C-D intact knee forces throughout the gait cycle (N=6)

Figure 28  A-P perturbations significantly affected A-P intact knee forces only during swing (N=6)
Figure 29  A-P perturbations significantly affected A-P ACL forces throughout the gait cycle (N=6).

Figure 30  ACL force-displacement curve (N=5). A representative force displacement curve to failure labeled with the corresponding maximum ACL resultant force during stance and swing when a simulated *in vivo* motion was applied (dotted lines). A detailed view of toe region is shown in a separate window.
ERROR: stackunderflow
OFFENDING COMMAND: ~

STACK: