

**A TRANSLATIONAL METHOD TO QUANTIFY
ANTERIOR CRUCIATE LIGAMENT HEALING
USING MAGNETIC RESONANCE IMAGING FOR
RESEARCH AND CLINICAL APPLICATIONS**

March 24, 2015

By

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BS, University of Connecticut, 2006

Thesis

Submitted in partial fulfillment of the requirements for the
degree of Doctor of Philosophy in Biomedical Engineering at Brown University

Providence, Rhode Island

May 2015

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2015

This dissertation by Alison M Biercevicz is accepted in its present form by the Department of Biomedical Engineering as satisfying the dissertation requirement for the degree of Doctor of Philosophy.

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Curriculum vitae

Ali attended the University of Connecticut where she attained a BS in Biomedical Engineering in 2006. After graduating from the University of Connecticut, Ali served as an Americorps Volunteer in Service to America (VISTA) in Helena, Montana. During her service term she worked on projects associated with law, lobbying and outreach to the poverty population through out the state. In 2007 she returned to the east coast, where she worked as a project manager for Rhode Island Hospital Orthopaedic Foundation (RIHOF), in Providence. During her time there she worked on a variety of engineering orthopaedic projects for industry and for Rhode Island Hospital Resident research. In 2010 Ali began work in the Bioengineering Lab within the Department of Orthopaedics at the Alpert Medical School and Rhode Island Hospital. Working with Dr. Braden C. Fleming, her dissertation adviser, she has been a part of an incredibly productive research team focused on the anterior cruciate ligament. As part of this research team, she has been an author on several peer-reviewed publications, conference proceedings, podium presentations, and poster presentations.

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EDUCATION

Brown University, Providence, RI
Department of Orthopaedics, Center for Biomedical Engineering and School of Engineering
PhD, Biomedical Engineering, April 2015

Adviser: Braden C Fleming, PhD

Thesis: A translational method to quantify anterior cruciate ligament (ACL) healing using magnetic resonance imaging (MRI) for research and clinical applications as a rehabilitation tool

University of Connecticut, Storrs, CT
Bachelor of Science, Biomedical Engineering, May 2006

Focus: Bio-materials and mechanics

SUMMARY OF CURRENT RESEARCH

Goals

- Develop a non-invasive method to quantify a healing ACL, or graft structural properties using MRI in an *ex vivo* animal model
- Adapt and optimize the MR data collection and prediction method for feasibility with a human clinical population
- Translate the MR strength prediction model to clinical, functional, and patient outcome measures obtained from an ACL reconstruction trial as an initial step to create a clinical tool to aid clinicians when determining the appropriate timing for athletes to return to sport
- Act as a member of a multifaceted research team, developing and evaluating the feasibility of ACL repair in a series of animal model biomechanical studies

Methods

- Collaborate with research scientists across disciplines at Brown University's Departments of Orthopaedics, Medical Imaging, and Computer Science to develop methodology for non-invasive prediction of strength of the healing ACL
- Assess MR imaging protocol for accuracy, repeatability, reproducibility, resolution, and time efficiency specific to soft tissue applications in a clinical setting
- Implement and analyze a series of *ex vivo* mechanical tests for evaluating surgical animal model and human cadaveric biomechanical performance
- Expand and optimize image processing software for creating MR outcome variables specific to modeling structural properties of a healing ligament
- Establish a series of integrated prediction models using statistical modeling from both animal model and clinical empirical data

GRANTS

National Institutes of Health (1R01-AR065462; Fleming/Murray), *Awarded*

Non-invasive assessment of ligament healing. \$1,175,000 (direct); 09/01/14-08/31/17, Multiple Principle Investigator (5th percentile rating)

Seed Grant- Awarded through the Department of Diagnostic Imaging, Division of Imaging Research, Rhode Island Medical Imaging

MRI measurement of knee ligament health in arthritis. \$15,000; 2013-2014.

PEER-REVIEWED PUBLICATIONS

1. Biercevicz AM, Proffen, BL, Murray MM, Walsh EG, Fleming BC. T₂* Relaxometry and Volume Predict Semi-Quantitative Histological Scoring of an ACL Bridge-enhanced Primary repair in a Porcine Model. Accepted by the *Journal of Orthopaedic Research*. February 2015.
2. Biercevicz AM, Rubin LE, Walsh EG, Merck D, Machan JT, Fleming BC. The Uncertainty of Predicting Intact Anterior Cruciate Ligament Degeneration in Terms of Structural Properties Using T₂* Relaxometry in a Human Cadaveric Model. Accepted by the *Journal of Biomechanics*. February 2015.
3. Biercevicz AM, Akelman MR, Fadale PD, Hulstyn MJ, Shalvoy RM, Badger GJ, Tung GA, Oksendahl HL, Fleming BC. MRI derived parameters of volume and signal intensity predict clinical, functional, and patient-oriented outcome measures following ACL reconstruction. *American Journal of Sports Medicine*. 2015;43(3):693-699.
4. Biercevicz AM, Walsh EG, Murray MM, Akelman MR, Fleming BC. Improving the clinical efficiency of T₂* mapping of ligament integrity. *Journal of Biomechanics*. 2014;47(10):2522-2525.

5. Biercevicz AM, Murray MM, Walsh EG, Miranda DL, Machan JT, Fleming BC. T₂ * MR relaxometry and ligament volume are associated with the structural properties of the healing ACL. *Journal of Orthopaedic Research*. 2014;32(4):492-9.
6. Biercevicz AM, Miranda DL, Machan JT, Murray MM, Fleming BC. In Situ, noninvasive, T₂*-weighted MRI-derived parameters predict ex vivo structural properties of an anterior cruciate ligament reconstruction or bio-enhanced primary repair in a porcine model. *The American Journal of Sports Medicine*. 2013;41(3):560-566.
7. Heard WMR, Paller DJ, Christino MA, Behrens SB, Biercevicz AM, Fadale PD, Monchik KO. Effect of Insertion of a Single Interference Screw on the Mechanical Properties of Porcine Anterior Cruciate Ligament Reconstruction Grafts. *The American Journal of Orthopedics*. 2013;42(4):168-172.
8. Kayiaros S, Biercevicz AM, Limbird RS, Paller D, Rubin LE. Broach Handle Offset and Impact Acceleration During Femoral Preparation for Total Hip Arthroplasty. *Reconstructive Review*. 2013; 3(2): 41-43.
9. Got C, Shuck J, Biercevicz AM, Paller D, Mulcahey M, Zimmermann M, Blaine T, Green A.. Biomechanical Comparison of Parallel Versus 90-90 Plating of Bi-column Distal Humerus Fractures With Intra-Articular Comminution. *The Journal of Hand Surgery*. 2012;37(12):2512-2518.
10. Daniels AH, Paller DJ, Feller RJ, Thakur NA, Biercevicz AM, Palumbo MA, Crisco JJ, Madom IA. Examination of cervical spine kinematics in complex, multi-planar motions after anterior cervical discectomy and fusion and total disc replacement. *The International Journal of Spine Surgery*. 2012;6(1):190-194.

RHODE ISLAND HOSPITAL OTHOPAEDIC MENTORSHIP PROJECTS

- Andrew Rohan, Candidate for B.S., Brown University. Quantitative histological processing for ligament integrity, Summer 2014 - Summer 2015.
- Mathew Shalvoy, Medical Student, Warren Alpert Medical School at Brown University. Cartilage thickness computation for comparison in ACL repair surgical model, Summer 2012 - Summer 2013.
- Christopher DiGiovanni, MD, Rhode Island Hospital Attending Surgeon. 3D Analysis of Ankle Stress Testing, Spring 2010.
- Stephen Kayiaros, MD, Rhode Island Hospital Resident. Broach Handle Curvature Effects Impact Acceleration During Broach Insertion for Hip Arthroplasty, Spring 2010.
- Steven Behrens, MD, Rhode Island Hospital Resident. Kinematic investigation of open ankle repair compared to arthroscopic repair due to blank ligament injury, Spring 2010.
- Daniel Aaron, MD, Rhode Island Hospital Resident. Mechanical evaluation of rat superspinatous repairs, Spring 2010.
- Mark Palumbo, MD, Rhode Island Hospital Attending Surgeon. Investigation of mechanism for trachea collapse post cervical spine surgery, Spring 2010.
- Keith Monchick, MD, Wendel Heard, MD, Rhode Island Hospital Residents. In vitro graft laceration and tensile strength of multiple soft-tissue anterior cruciate ligament reconstruction techniques in a porcine knee model, Fall 2009-Spring 2010.

- Chris Got, MD, Rhode Island Hospital Resident. In vitro fixation strength of two distal humeral fixation techniques, Fall 2009.
- Nikhil Thakur, MD, Rhode Island Hospital Resident. Stability of intertrochanteric fractures treated using intramedullary nailing, Fall 2009-Winter 2010.
- Peter Fitzgibbons, MD, Rhode Island Hospital Resident. Laxity in the knee following arthroscopic meniscal root repair, Spring 2009-Summer 2009.
- Wendel Heard, MD, Rhode Island Hospital Resident. Comparison of the mechanical and the anatomical axis of the wrist, Spring 2009.
- Eve Hoffman, MD candidate. Characteristics of neutral cushioning running shoes during long term fatigue, Winter 2008-Spring 2009.

SPECIFIC RESEARCH EXPERIENCE

Mechanical Testing

Servohydraulic and electric: Quasi-static and dynamic test procedures on Instron, MTS and Bose test frames; Design of mechanical test procedures for both biological tissue and medical devices; Fabrication of test fixtures for specific biological applications

Animal Model Surgery

Surgical preparation: Administered IVs, intubation, sterile field preparation; Induced and monitored anesthesia and analgesia

Survival procedures: Rat nephrectomy, thyroidectomy, parathyroidectomy, sciatic nerve guidance channel placement, aorta graft anastomosis; Mouse renal subcapsular implants; Ferret ovariectomy; Pig small bowel resection; Rabbit carotid vascular repair

Human Cadaveric Model

Rhode Island Hospital Orthopaedic resident anatomy conferences: Spine, hip, knee, foot, shoulder, elbow and hand

Anatomical dissection: Human cervical spine, thoracic spine, lumbar spine, knee, foot, shoulder, elbow and hand cadaveric models

Assisted surgical procedures: Vertebroplasty; Spinal fusion with facet screws, pedicle screws, anterior plating, and cages; ACL reconstruction; Meniscal root repair; Resurfacing of the knee, shoulder and great toe; Arthroscopy in the shoulder, wrist, hip and knee; Tendon harvests of semitendinosus, gracilis, patellar tendon with bone block

Microscopy

Bright field and polarized light: H&E staining for quantifying cellular scoring; Picrosirius red staining and collagen specific anti-bodies for collagen content assessment

PROFESSIONAL EXPERIENCE

Rhode Island Orthopaedic Foundation, Providence, RI

Project Leader

October 2007 - June 2010

- Designed and implemented testing methodology for Brown University and Rhode Island Hospital Orthopaedic Resident research projects
- Conducted mechanical testing with servo-hydraulic test frames for American Society of Testing and Materials (ASTM) standards and material properties analysis
- Functioned as a creative member of an engineering team, developing innovative orthopaedic products for FDA approval
- Utilized 3D motion capture technology for a variety of orthopaedic research projects including spinal and femoral kinematics
- Managed US and International Women's lacrosse tests and carried out American Association for Laboratory Accreditation (A2LA) procedures

Montana Legal Services Association, Helena, MT

Americorps Volunteer in Service to America (VISTA)

July 2006 - July 2007

- Coordinated state-wide awareness and outreach for poverty law issues
- Created and implemented a state-wide customer service evaluation
- Conducted a case study with clients on a variety of legal issues

Unilever Home and Personal Care, Trumbull, CT

Process Development Engineer

May 2005 - January 2006

- Developed manufacturing processes for large scale Dove brand production
- Analyzed data for ultrasonic equipment development in a pilot plant environment
- Oversaw and planned project for production simplification

HONORS AND AWARDS

Orthopaedic Research Society podium presentation, February 2013

Spot light session: Orthopaedic Research Society presentation in the Injured and Diseased Tendon Session. "Non-invasive Prediction of Healing Ligament Structural Properties with T_2^* and Volume."

Orthopaedic Research Society podium presentation, January 2012

Spot light session: Orthopaedic Research Society presentation in the ACL Reconstruction Session. " T_2^* Weighted MRI Derived Morphology and Signal Intensity to Determine Structural Properties of an ACL Reconstruction Graft in a porcine model."

Brown University Travel Award, March 2015, March 2014, February 2013, January 2012

Granted through the Department of Biology and Medicine and the Center for Biomedical Engineering

CONFERENCE PROCEEDINGS

- First Author Poster Presentation, *MRI Derived Parameters Of Volume And Signal Intensity Predict Clinical, Functional And Patient-oriented Outcome Measures Following ACL Reconstruction*. Orthopaedic Research Society, 2015.
- First Author Poster Presentation, *T_2^* mapping the intact PCL: Can we improve computational time with relaxometry post-processing?*. Orthopaedic Research Society, 2014.
- First Author Poster Presentation, *T_2^* Weighted MRI Derived Morphology and Signal Intensity to Determine Structural Properties of an ACL Reconstruction Graft in a Porcine Model*. Gordon Research Conference, 2012.
- Co-Author Podium Presentation, *Airway Obstruction after Anterior Cervical Spine Surgery: Hematoma Creates Tracheal Compression within Physiological Pressures*. Cervical Spine Research Society, 2010.
- Co-Author Poster Presentation, *Influence of Laceration and Interference Screw Fixation on Tensile Strength of Soft-Tissue Grafts*. American Academy of Orthopaedic Surgeons, 2011.
- Co-Author Poster Presentation, *Cervical Spine Stiffness Following Total Disc Replacement Mimics Intact Behavior in Primary and Coupled Motions with Flexion/Extension Components*. Orthopaedic Research Society, 2010 .
- Co-Author Poster Presentation, *Bending and Hybrid Vertebroectomy Models for the Assessment of Fusionless Scoliosis Growth Rods*. Orthopaedic Research Society, 2010.
- Co-Author Poster Presentation, *Characteristics of Neutral Cushioning Running Shoes during long term fatigue*. Rhode Island Hospital Annual Research Celebration, 2009.

TEACHING EXPERIENCE

Brown University, Providence, RI

Graduate Research Mentor

May 2010 - Present

- Mentor undergraduate, medical students, and orthopaedic residents during independent research

Summer at Brown, Providence, RI

Course Instructor: "Do you want to be an Engineer?"

April 2011 - August 2014

- Compiled, organized, and presented lectures exposing international high school students to engineering fundamentals in preparation for a collegiate experience
- Worked independently and in collaboration with fellow instructors to differentiate course material for a range of student abilities and educational backgrounds
- Conducted interactive labs involving problem solving, mechanical engineering, biomedical engineering, and chemical engineering topics

Sheridan Center, Providence, RI

Certificate I: Seminar on Reflective Teaching

September 2013 - May 2014

- Awarded certificate for completion of seminar and workshop curriculum on developing and refining fundamental teaching and assessment strategies as well as communication skills

SKILLS

Software: MATLAB, SolidWorks, Visual3D, Materialise Mimics, Geomagic Studio, LyX, Image J, Brown University Center for Computation and Visualization, Sigma Plot, Adobe products

Hardware: Magnetic Resonance Imaging (MRI); 3D Motion Capture; Instron, MTS and Bose servo-hydraulic, single axial, bi-axial, and continual torsion test frames; Computed Tomography (CT); Dexa Scanning (bone mineral density); C-arm Fluoroscope; Data Acquisition

Additional: Modified human subject research protocols and consent forms according to Institutional Review Board (IRB) standards; Assisted and implemented recruitment and consent of human subjects; Assisted with human subject research

RESEARCH AND DEVELOPMENT INTERESTS

Biomechanics, Sports Injury, Anterior Cruciate Ligament (ACL), Kinematics, Kinetics, Osteoarthritis, Injury Prevention, Injury Rehabilitation, Disease Progression, Medical Imaging, Image Processing, Biomechanical Modeling, Tissue Engineering, Mechanical Testing, Orthopaedic Medical Devices, Prosthetics, Product Design, Product Development, Data Collection / Processing / Analysis

SOCIETAL AFFILIATION

Orthopaedic Research Society (ORS), 2011 - Present

Preface and acknowledgements

My last five years at Brown University have been the most challenging and rewarding of my life so far. When I started my PhD 5 years ago, I knew transitioning back to student life would be challenging; however, this process was made enjoyable due to the unique environment of the Biomedical Engineering Department. I have really enjoyed every minute of my time as a student here and all of the opportunities that have been afforded to me. I would specifically like to acknowledge the support of the Rhode Island Orthopaedic Department and of the Biomechanics Lab. I have called suite 404 a second home for many years and that is due to the people and friendships. Every single person I have worked with has left a lasting impression on me and been integral to my success. I truly appreciate every member of the family here, past and present. I only hope I am afforded the opportunity to work with them for many years to come.

I also need to thank the Brown MRF community. When my research project first started I never thought I would get the opportunity to learn so much about the field of MRI. I have loved every minute of this learning experience and I have many members of the MRF community that have taken me under their wing to thank for that.

Dr. Braden Fleming has been a pleasure to work with every day of my PhD experience. I met Braden well before becoming a student, when I worked as an engineer for Rhode Island Orthopaedic Foundation and did the mechanical testing for Braden's long-term pig studies. I will never forget the day when a well-timed joke led to a 5 year PhD program. It is very difficult to summarize in words how lucky I have been to have his guidance, not only as a mentor but also as a life coach. I have learned so much under his apprenticeship; from putting together a study methodology to the fine points of composing a well-worded email. I also have him to thank for developing a great deal of perseverance and patience. His support through the many hours of coding my project required, were integral to my success. I would also like to mention his support with developing my teaching skills through Summer at Brown. I am

extremely appreciative of his support in this capacity and I am a very well rounded student as a result. As I complete my PhD, I am struck by the realization that while I am very happy to be finishing this chapter of my life, I am saddened by the fact that it will no longer be working for him.

I would also like to thank my thesis committee. Trey Crisco has been incredibly supportive as another mentor in the biomechanics lab. Having him on my committee has been crucial in shaping the direction of the project and its success. Eric Darling has been extremely supportive not only as a department head but with my individual research. I really appreciate all his time and effort over the past 5 years. Sean Deoni has not only been a mentor and a teacher but a friend. After having taken his Magnetic Resonance Imaging class my first semester at Brown, I have never looked back. He has been a huge inspiration in my efforts to apply the field of MRI in orthopaedics. Gail Thornton has been a huge asset as my project has developed. Her perspective has influenced my project greatly and for that I am extremely grateful.

I would like to thank my Mom and Dad not only for their support through out the PhD process but for their support the last 30 years! I cannot say how lucky I have been to have them as parents and for supporting my math and science interests from a very early age. Also to my sister, you have been an inspiration to me as early as I can remember, thank you for paving the way for engineering at UConn, even if a professor or two may have remembered you and given me “special” treatment as a result. Also to my brother in-law and my amazing niece and nephew thank you for being a loving family. I also must thank my boyfriend for all the support and sacrifice over the past few years. There have been many days where I have stopped to think how lucky I am to share my life with such an amazing man. Much of this wouldn't be possible without his support.

Finally, where would I be without my friends? I have gotten insanely lucky with the life long friendships I have developed in the past years. Every single one of you has been a source of joy, support and adventure.

Dedication

I dedicate this to my amazing boyfriend and best friend, Noel. It is his love, support and laughter that has made this possible.

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Nomenclature

ACL	Anterior Cruciate Ligament
ACLT	ACL transection
BE-ACL	Bio-enhanced ACL repair
CI	Confidence Limits
CISS	Constructive interference in steady state; T2* weighted MR imaging sequence (Siemens, true FISP)
DICOM	Digital Imaging and Communications in Medicine
TE	Echo time- MR image acquisition parameter
M0	Equilibrium magnetization
FOV	Field of View
FLASH	Fast low angle shot; T1 weighted MR imaging sequence (Siemens, spoiled gradient echo)
FA	Flip angle- MR image acquisition parameter
IKDC	International knee documentation committee score
KOOS	Knee injury and osteoarthritis outcome score
KT-1000	Knee arthrometry measurement device and test
MGV	Median Gray Value- subjective units for SI
MRI or MR	Magnetic Resonance Imaging

Q1	First quartile summary statistic
Q2	Median- 2nd quartile summary statistic
Q3	Third quartile summary statistic
RF	Radio Frequency
TR	Repetition time- MR image acquisition parameter
SI	Signal Intensity
SNQ	Signal to noise quotient- subjective units for SI
SNR	Signal to noise ratio
SE	Standard Error
T1	T1 relaxation time- spin lattice relaxation
T2	T2 relaxation time- spin spin relaxation
T2*	T2* relaxation time- similar to T2 relaxation time but is accomplished with gradient echo imaging (instead of spin echo as in T2 images). T2* reflects not only true T2 relaxation, but also the effects of magnetic susceptibility gradients at both a macroscopic and microscopic level.
3D or 3-D	Three dimensional
2D or 2-D	Two dimensional
VOL or Vol	Volume
VW	Volume of the whole ligament
WSCV	Within subject coefficient of variation

Chapter 1

Introduction

Overview

The rate of anterior cruciate ligament (ACL) injury has recently been reported to be as high as 400,000 cases per year in the United States.[27] Unfortunately, graft failure[13,48] and post-traumatic osteoarthritis (OA)[11,22,32,41,43,44] are common complications following ACL reconstruction (a surgical procedure in which the injured ligament is replaced with a tendon graft). Due to these complications, research has been focused on developing new, more effective methods of treatment, including bio-enhanced ACL primary repair (a surgical procedure in which the injured ligament is repaired using sutures and a biologically active scaffold).[18,37,39,56] Current clinical, functional and patient oriented outcomes for evaluating the success of different ACL treatments include knee arthrometry, hop testing and patient assessment surveys.[6,17,47,53] However, most of these indirect methods are not sensitive enough to detect structural changes of the graft or ligament itself.[1,3,35] As a result, *ex vivo* animal models have been used to determine the structural properties of a reconstructed graft as an outcome measure for evaluating graft strength and documenting healing.[18,39] However, this approach requires destructive testing and is not suitable for longitudinal *in vivo* use with animal models or clinical assessment. This makes a reliable, quantitative, *in vivo*, method for determining the biomechanical performance of the ligament or graft during healing highly desirable in both a research and clinical setting.

Magnetic resonance (MR) imaging is already widely used for qualitatively monitoring ACL graft health following surgical reconstruction in a clinical setting. Furthermore, MR's ability to measure the geometry of anatomic structures,[4,5] such as the ACL, and provide information about tissue water content and organization makes it an ideal choice for *in vivo* assessment of a graft following reconstruction. [2,30,31] A non-invasive MR method for predicting the structural properties of a graft or ligament would allow researchers to document functional healing in pre-clinical animal studies and clinical trials.

The long-term objective of this study was to develop and implement a non-invasive MR based technique to evaluate the structural properties of the healing ACL or graft following treatment. The first four specific aims address the design of an MR imaging method to predict the structural properties of a healing ligament or graft in a large animal model. This method was also used to assess microscopic outcomes of a healing ligament in the terms of histological analysis. After establishing the prediction method in an animal model, it was translated in the remaining two aims to the intact human ACL as well as to clinical, functional, and patient-oriented outcome measures following ACL treatment.

1.1 Background

1.1.1 Anterior Cruciate Ligament (ACL) Injury:

As one of two cruciate ligaments located at the center of the knee, the primary function of the anterior cruciate ligament (ACL) is to provide stability to the joint during functional movements (Figure 1.1).[42] Unfortunately, the ACL is often injured or torn during non-contact activities involving cutting, pivoting, or sudden deceleration maneuvers.[29] To treat ACL injuries the current gold standard is ACL reconstruction utilizing patellar tendon or hamstring tendon autografts.[23] However, abnormal knee kinematics[55] and graft failure[13,48] are common complications of this procedure. This has prompted research exploring a variety of novel approaches for treating ACL injury, both in animal models and clinical studies, in hopes of improving patient outcome.

1.1.2 Current alternative ACL treatment research:

One novel alternative treatment to reconstruction, that has shown promise, is bio-enhanced (or bridge-enhanced) ACL primary repair.[39,56] Instead of replacing the native ligament with a graft, the natural ligament is preserved in what is called primary repair. The ends of the torn ACL are stitched together with suture and a bio-active scaffold is positioned over the ligament to help with clot formation and to promote healing. In cross-sectional animal models, bio-enhanced primary repair has shown positive histological and biomechanical results in comparison to standard graft reconstruction.[18,39]

1.1.3 Histological Assessment of ACL healing:

Histological scoring is one semi-quantitative modality used to evaluate ligament healing following reconstruction or bio-enhanced repair on a microscopic level.[37] Hematoxylin and eosin (H&E) staining is one common histological method that has been used to estimate the ligament maturity index score, a cumulative measure of ligament cell count and collagen fiber dimensions that is sensitive to tissue healing and has been found to correlate to structural properties.[45] Picrosirius red is another stain that has been used in conjunction with circularly polarized light microscopy to assess tissue healing in terms of collagen fiber size and density.[12,28,49] Histological methods using picrosirius staining can also detect collagen degradation or pathology[59] and could allow researchers to further assess ligament response

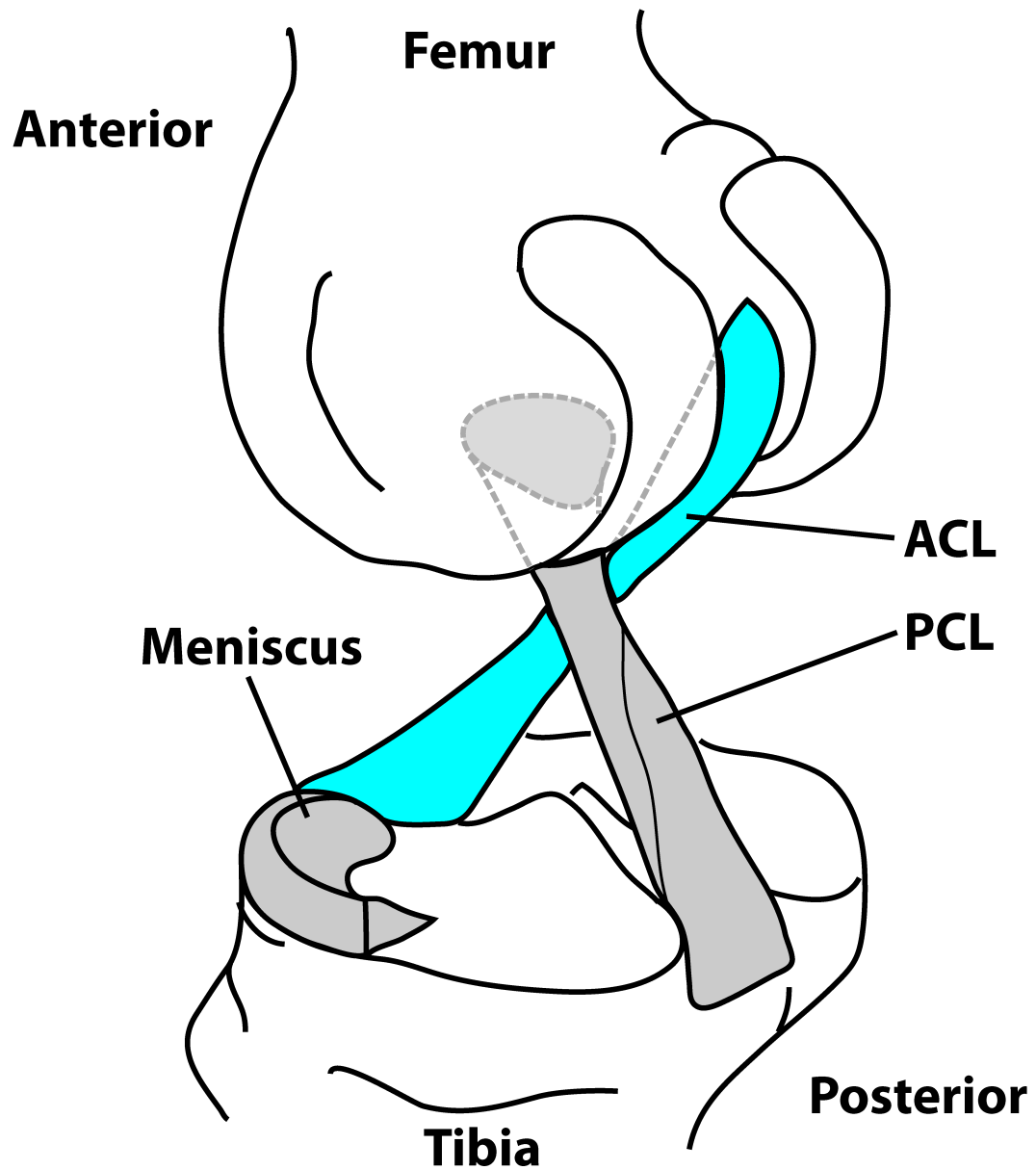


Figure 1.1: Schematic showing an expanded posterior view of the knee joint. The anterior cruciate ligament and posterior cruciate ligament are located at the center of the joint and connect the femur to the tibia and provide stability during functional movements. Figure adapted from Keil, D., Yoganatomy The Knee Part 1 - Working with the knee in yoga postures, <http://www.yoganatomy.com/2011/10/the-knee-part-1-by-david-keil-2005-enlightened-practice-magazine/>. accessed February 2014.

on a micro-scale. While histology is an effective means to assess healing, it is inherently limited by its semi-quantitative nature and its reliance on invasive *ex vivo* sample preparation.

1.1.4 Biomechanical Assessment of Ligament healing:

Inherently quantitative biomechanical measures, such as structural properties, are ideal for evaluating strength and documenting healing of the native ACL or reconstructed graft.[7,20,38,40,57] Currently, the only way to assess the structural properties of a healing ligament is with *ex vivo* biomechanical testing. Structural properties serve to assess a ligament's collagen response to quasistatic loading in the terms of maximum load, yield load and linear stiffness (Figure 1.2).[42]

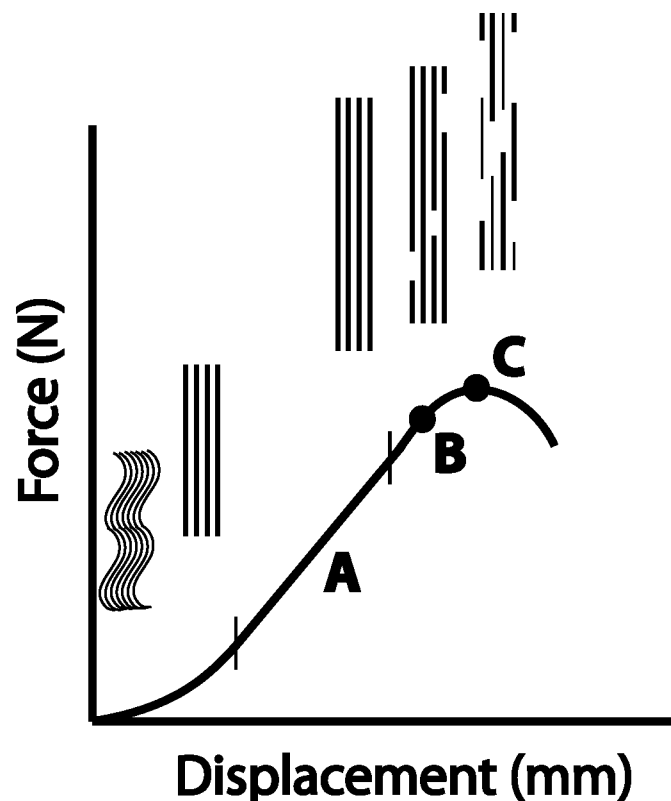


Figure 1.2: Example Force displacement curve for a ligament's collagen fiber response to loading. A. Slope of the line represents linear stiffness. B. Change in slope represents yield load. C. Maximum load. Figure adapted from Frank CB, Shrive NG. Ligament (2.5). In: Nigg BM, Herzog W, eds. Biomechanics of the musculoskeletal system, 2nd ed. New York: John Wiley & Sons, 1999:107-126.

Previous research has determined the structural properties of intact, reconstructed, and repaired ligaments by utilizing a destructive tensile testing protocol and isolating the femur-ligament-tibia complex from other joint soft tissues (Figure 1.3).[20,38,57] From these studies, a strong correlation has been

found between structural properties and healing following ACL repair or reconstruction,[20,38,57] making structural properties a useful proxy for graft healing. Furthermore, the quantitative nature of the structural properties makes it an ideal measure for directly comparing different reconstruction or repair treatments.

Despite being a useful quantitative measure of graft healing, determining structural properties with this method requires destruction of the joint for testing. Therefore, pre-clinical studies of traditional and novel technologies require large numbers of animals that must be sacrificed at each healing time point (i.e., cross sectional study) when the structural properties are measured. In addition, as there is significant variability in the biologic response to injury and repair even across same-strain animals, relatively large numbers of animals must be used at each time point to provide sufficient power for detecting significant differences between treatment groups. Use of a non-invasive technique for the prediction of the biomechanical properties of a healing ligament would allow researchers to perform longitudinal studies of individual animals (over multiple time points of interest). Thus it could lower the number of animals required per study group and significantly lower the study cost. In developing novel treatment options, a method to non-invasively assess the healing ACL or graft would greatly improve the efficiency of these types of animal model for *in vivo* pre-clinical trials. Furthermore, these findings may have implications as a surrogate outcome measure in clinical studies for documenting temporal changes within-patients.

1.1.5 Current clinical assessment of ACL healing:

A new non-invasive strategy to evaluate ligament healing would also significantly improve the efficiency of clinical trials for comparing treatment options. Currently, clinical, functional, and patient-oriented outcome evaluation techniques, such as knee arthrometry (KT-1000)[6], hop testing, and patient oriented outcome forms (IKDC- International Knee Documentation Committee Examination score and KOOS- Knee Osteoarthritis Outcome Scores), have been useful in many clinical studies as a standardized way to evaluate patient outcome following ACL treatment.[10,17] Knee arthrometry, which measures laxity of the entire knee joint, is an example of a clinical outcome measure that has been widely used;[53] however, because it is an indirect measure of graft or ligament laxity it requires large numbers to achieve significant power and detect differences in treatment groups. An example of a functional outcome test is the single leg hop test, which compares the distance jumped by a patient with their operative knee to the distance jumped with their intact knee (Figure 1.4). This test has been cited as a practical functional outcome measure, that reflects the effects of knee strength as well as the patient's confidence in the

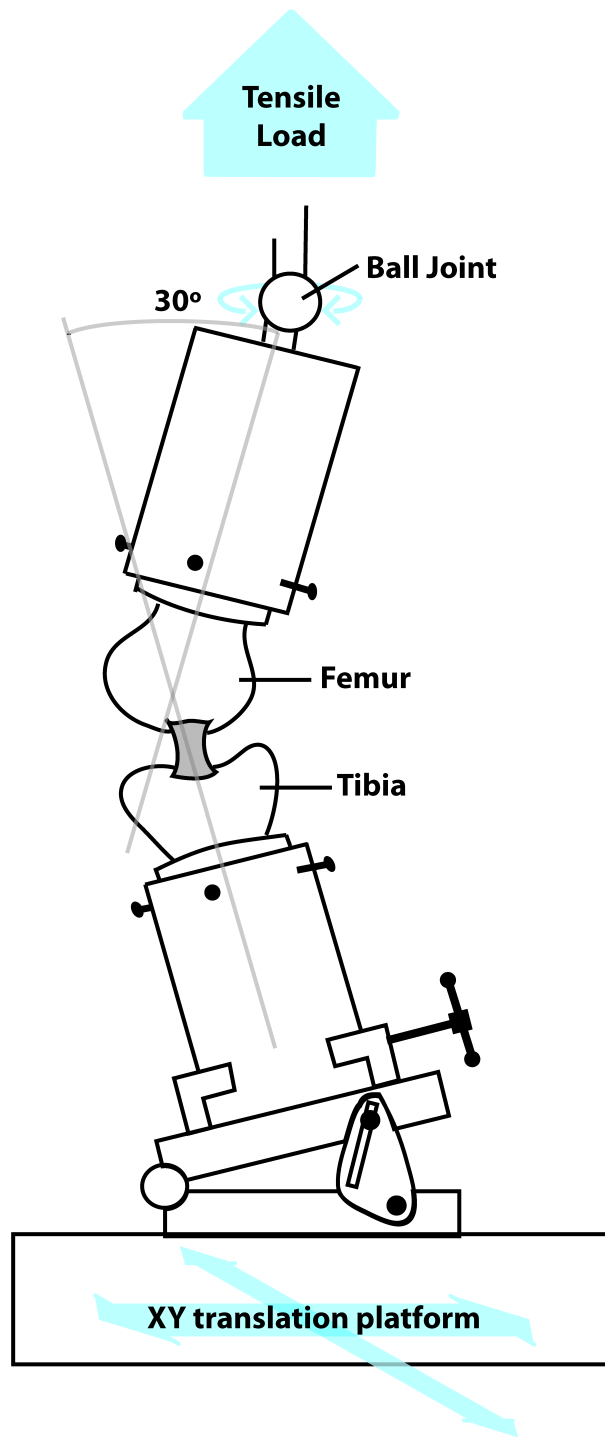


Figure 1.3: Schematic showing an example tensile test setup. The isolated femur-ligament-tibia complex is in the center with a ball joint and X-Y translation platforms to ensure proper alignment of the ligament with the direction of load application throughout the duration of the test. Figure from Fleming, B. C., Spindler, K. P., Palmer, M. P., Magarian, E. M., and Murray, M. M., Collagen-platelet composites improve the biomechanical properties of healing anterior cruciate ligament grafts in a porcine model, *Am J Sports Med* 37 (2009) 1554–1563.

operative limb.[47,58] However, this test, similar to knee arthrometry, focuses on the functionality of the entire knee and not that of the healing graft or ligament itself. Questionnaire-based patient-oriented outcome measures, such as the IKDC or KOOS, face similar issues with specificity and power and as such require large sample sizes.[9,15,54] The lack of sensitive outcome measures for clinical studies may be one reason why no improvements have been found in many clinical trials comparing outcomes of different ACL reconstruction techniques (e.g., comparisons of graft type,[35] graft position,[1] rehabilitation,[3] or graft tension[17]).

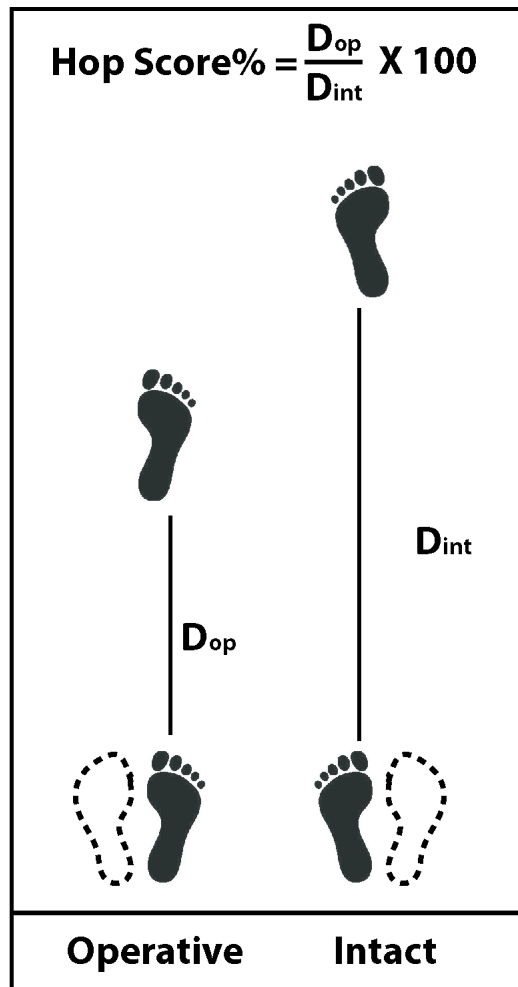


Figure 1.4: Schematic showing the single leg hop test where a patient hops for distance on both the surgical and intact legs. The patient hop score is calculated as percentage of the contra-lateral limb.

While these traditional clinical, functional, and patient oriented outcomes have been useful in a clinical setting they have limitations and they are difficult if not impossible to implement on animal models. Furthermore, traditional patient outcome measures are not specific enough to detect differences in the structural properties of a healing ligament (i.e., tensile failure properties) between treatment groups.

Thus, a more specific quantitative method for evaluating potential treatments is desirable for both preclinical animal model research and clinical trials.

1.1.6 Magnetic Resonance imaging as an alternative evaluation of ligament healing:

An alternative to these traditional clinical and *ex vivo* methods is magnetic resonance imaging (MRI), which is a non-invasive, information dense modality, that has the potential to quantify ACL treatment outcome. MRI is already widely used by clinicians to qualitatively and noninvasively monitor ACL graft health following a reconstruction.[33,34] With 3T strength magnets widely available,[52] MR can be used to measure the geometry of complex structures, such as the ACL.[4,5] In addition, MR imaging techniques are constantly evolving and have the potential to provide information about tissue on a microscopic level by using specific sequences and post processing to determine tissue water content and organization.[21] Signal intensity, an MRI parameter that is a function of tissue type as well as water content, has been used to evaluate ACL graft integrity and maturation following reconstruction surgery.[16,24,25,36,46,51] Using semi-quantitative clinician graded scores based on MR signal intensity, ACL treatment potential has been evaluated in patient populations.[16,26,36,46,51] More specifically, signal intensity has been used to compare two ACL reconstruction treatment groups, with and without autologous platelet concentrate, [16] and to evaluate the graft in bone tunnels following reconstruction.[36] While useful in a research context, signal intensity in this case is limited by the natural subjectivity of clinician grading and also because signal intensity is dependent on MR scan parameters as well as scan type and is not a fundamental property of the tissue.[14]

1.1.7 Signal Intensity correlation with ACL healing:

The use of graft signal intensity (SI) observations for clinician graded scoring, is based on research showing that graft signal intensity decreases with time post-operatively.[51,57] In an ovine (sheep) study, T_1 weighed images were used to analyze the signal intensity to noise quotient (SNQ) of a single mid-substance slice of a reconstructed graft at 6, 12, 24, 52 and 104 weeks post-operatively. SNQ was found to inversely correlate to structural properties, with grafts of low SNQ exhibiting the highest structural properties (Figure 1.5). It was also found that lower SNQ correlated with histological confirmation of the healing process further indicating that SNQ could serve as marker of graft maturation and remodeling.

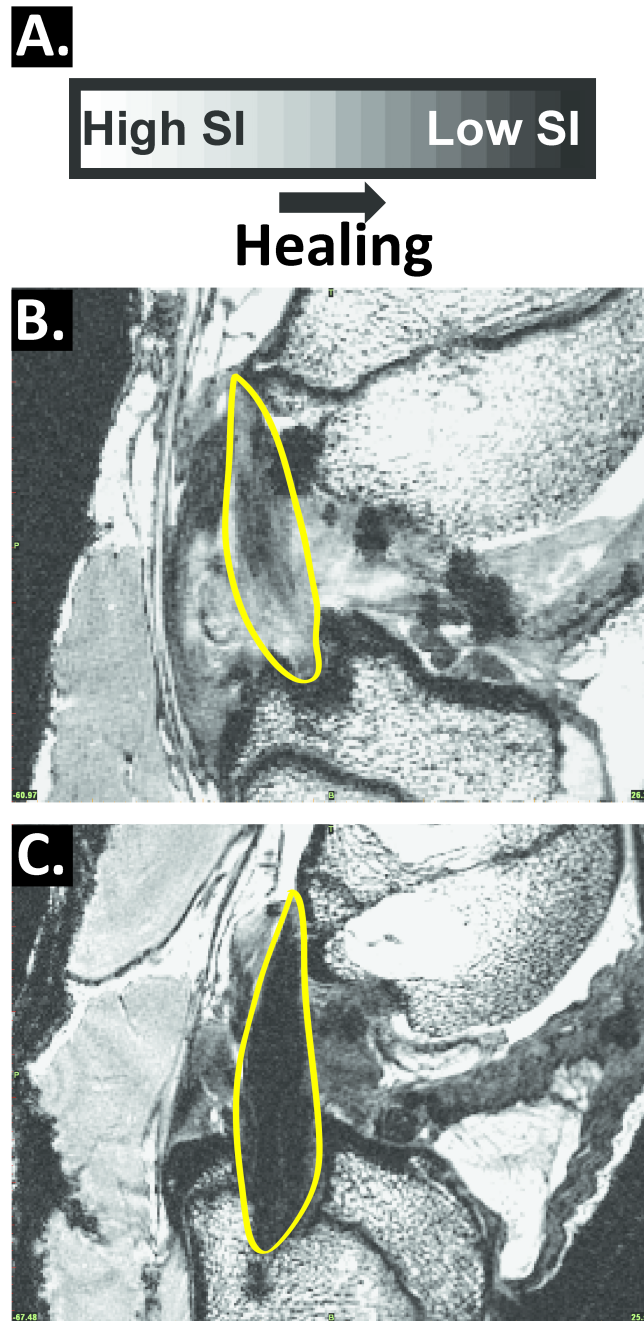


Figure 1.5: SI is correlated to graft or ligament healing A. High SI is related with low structural properties and early time points in healing, while low SI is related to high structural properties and late time points in healing. B. Example high SI ACL reconstruction graft. C. Example low SI ACL reconstruction graft.

The direct comparison of *in vivo* imaging results to *ex vivo* testing are important findings with clinical implications.

Limiting the impact of this study, no correlation between geometry of the specimens and structural properties was evaluated, thus making comparison between specimens or patients difficult. Additionally, MR images are typically scaled for display according to the highest pixel intensity and because ligaments typically have low signal intensity due to their ultra structure this makes assessment of small signal differences unreliable. Finally, the use of signal intensity as an outcome measure is limited by its dependence on image acquisition parameters and scanner manufacturer, rendering the predictions to be protocol, magnet, and hence, institution specific. While SI has successfully been used as a quantitative measure, a less subjective metric would be ideal for assessing ligament integrity *in vivo*.

1.1.8 Volume normalized to T₂ relaxometry predicts structural properties:

One way to standardize MR results between scanners and imaging protocols is to use relaxation time variables, such as T₂ and T₂*. These relaxometry variables are inherent tissue properties, that reflect specific tissue characteristics (ultrastructure or nanostructure), and are much less sensitive to image acquisition parameters than conventional signal intensity data.[14] In a recent relaxometry study, it has been established that graft volume when measured *in situ* via MR imaging also correlates with graft structural properties. It was also found that this correlation could be improved by normalizing the graft volume to the MRI signal intensity derived parameter T₂ relaxation time (T₂), collected three months post-operatively in the caprine model.[19] T₂ was calculated by fitting the signal intensity of a series of scans with increasing echo time (TE) according to the following equation.

$$SI(TE) = M_0 e^{-TE/T_2} \quad (1.1)$$

Where SI(TE) equals signal intensity at varying echo times, M₀ equals base level magnetization, TE equals echo time and T₂ equals T₂ relaxation time.

While it is important that this study found a correlation between volume normalized to T₂ and structural properties, the study did not find a significant correlation between structural properties and T₂ values

as a separate quantifiable variable. This finding could be attributed to the single 6 week time point investigated with this study, which would have limited the range T_2 values to one phase of graft healing. Furthermore, the T_2 values in this study were determined using 2D mid substance MRI slices of the graft. This could result in error with consistently locating a “mid-substance” slice of a relatively small structure, such as the ACL, with this 2D method. Additionally, collecting T_2 traditionally requires relatively long echo times and maybe too long for gathering relaxation times of ligament or tendon tissue ($<10\text{ms}$).[50] A non-invasive approach to quantifying a metric defined by two graft characteristics (volume normalized to T_2) and how this metric affects structural properties is significant; however, this study was limited by the insignificant correlation of T_2 alone to structural properties. With further development, a more specific imaging methodology can be established for use with a healing ACL or graft.

1.1.9 T_2^* relaxation time to assess tissue integrity:

T_2^* relaxation time is another MR parameter that is available for evaluation of innate tissue properties. T_2^* is similar to T_2 but traditionally utilizes shorter echo times giving T_2^* the ability to capture tissues with short relaxation times (Figure 1.6).[8,59]

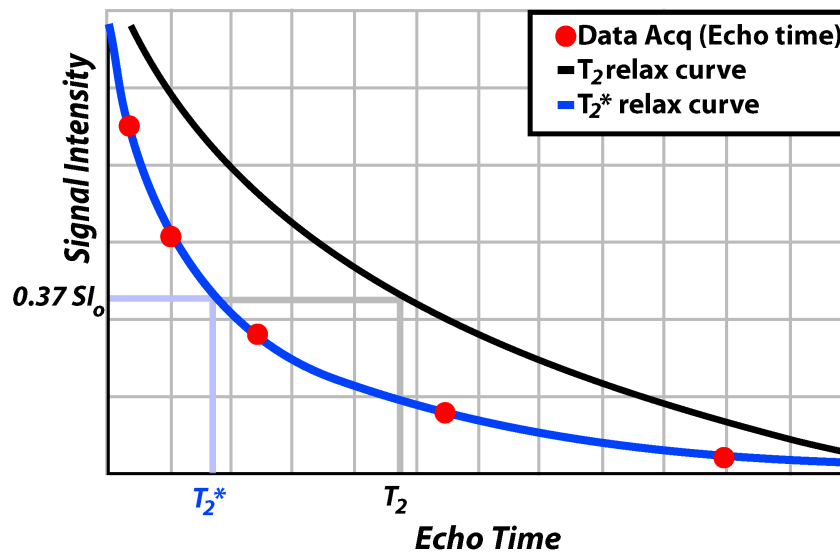


Figure 1.6: Theoretical relaxation decay curves fit to SI at varying echo times. Note the faster decay of T_2^* in comparison to T_2 relaxation. This allows T_2^* to capture more organized tissues with shorter relaxation times. Figure adapted from Chavhan, G. B., Babyn, P. S., Thomas, B., Shroff, M. M., and Haacke, E. M., Principles, Techniques, and Applications of T_2^* -Based MR Imaging and Its Special Applications, Radiographics 29 (2009) 1433–1449.

Scans that are T_2^* weighted are particularly good at musculoskeletal imaging due to optimized contrast between joint structures and joint fluid.[33] T_2^* reflects not only true T_2 relaxation, but also the effects

of magnetic susceptibility gradients at both a macroscopic and microscopic level. It has been shown that the magnetic susceptibility of ligaments varies with water content.[2] This variation is due to the interaction of water with collagen (residual dipolar coupling, susceptibility anisotropy and bulk magnetic susceptibility) where water mobility is restricted and this constraint causes rapid relaxation of the MR signal following excitation. This effect accounts for the short T_2^* in ligaments and other structures with highly organized ultrastructure and an abundance of type I collagen.[59] The correlation of T_2^* to tissue structure has made it ideal for detecting subclinical meniscal degeneration in a human cadaveric model.[59] A significant link was found between histological evidence of tissue degradation and increased T_2^* that would be expected with less organized tissue.[59]

With a healing ligament or graft a process opposite of tissue degradation occurs. Ligamentous tissue becomes more organized, as fibrils form and the graft remodels,[42] which should result in a decrease in T_2^* relaxation time. The link between T_2^* relaxation time and tissue ultrastructure found in meniscus research, could make T_2^* an ideal measure of graft healing and, when used in conjunction with graft volume a viable prediction model for structural properties.

1.2 Significance

To date there have been no studies evaluating the quantifiable MR parameters of volume, signal intensity and T_2^* relaxation time to predict the biomechanical or histological outcomes of an ACL graft or ACL repair as a surrogate outcome measure for healing. The contents of this thesis focus on developing imaging methods for determining these MR parameters and evaluating how these parameters can be used for assessing ligament or graft healing, in both animal models and in clinical trials. Additionally, a complimentary study determines if these imaging parameters are associated with biomechanical performance of the intact human ACL in cadaveric specimens. This part of the thesis provides a translational link between animal model data and clinical use. With the completion of the aims of this study, we have developed an MR imaging technique tailored to the healing ligament or graft and assessed its ability to be used as a quantitative outcome measure of ACL treatment.

A non-invasive MR method for predicting the structural properties of the graft or ligament would allow researchers to document functional healing longitudinally within a specimen in pre-clinical animal studies. This prediction method will be advantageous for evaluating results non-invasively at early time points, thus removing the need for euthanasia and mechanical testing at those time points and reducing the

number of animals and resources required for a study. Furthermore, these findings may have implications as a surrogate outcome measure in clinical studies for documenting temporal changes within-patients and as a more quantitative method for guiding rehabilitation and determining when a patient is ready to return to sport.

1.3 Specific aims

The long-term objective of this study is to develop and implement a non-invasive MR based technique to evaluate the biomechanical or histological outcomes of the healing ACL or graft following treatment in a large animal model and ultimately in a clinical setting. Six specific aims have been designed to investigate the efficacy of potential MR imaging methods to predict healing of a ligament or graft and to see how these methods relate to the intact human cadaveric ACL as well as clinical, functional and patient outcome measures.

1.3.1 Specific Aim 1

Develop a method to predict the structural properties of a healing ligament (ACL primary repair) and graft (ACL reconstruction) in a porcine model using the MRI derived parameters of volume and signal intensity (SI).

Our objective was to use a long-term porcine model to create 3D models of reconstructed and bio-enhanced repaired ACLs, using MR images, to determine ligament volume and signal intensity. We then evaluated the relationship of these MR variables to ligament structural properties, determined using an *ex vivo* tensile testing protocol. We hypothesized that graft or ligament volumes and signal intensity values, would be significant predictors of their structural properties after 15 weeks and 52 weeks of healing. Additionally, we hypothesized that the combination of volume and SI would offer a more complete evaluation of graft biomechanical performance than either variable alone.

1.3.2 Specific Aim 2

Develop and test a method to collect T_2^ relaxation time as a universal MR outcome that is tailored to imaging a healing ligament or graft. A secondary aim of this study was to compare the ability of*

the T_2^ relaxation time parameter to predict structural properties to that of the previous signal intensity parameter (Specific Aim 1), with the intent of determining the relative reliability of the measures.*

To address this aim we used a multi-echo imaging protocol for T_2^* determination, to collect the expected range of T_2^* values for a healing ligament. With this protocol we collected MR images of bio-enhanced repaired ACLs in an animal model (Specific Aim 1) and determined ligament volume, signal intensity and T_2^* values. We hypothesized that a multiple regression model based on ligament volume and its corresponding T_2^* values would predict the ligament's structural properties. We also hypothesized that the coefficients of determination would be greater and that the standard errors would be less when using the T_2^* prediction method compared to the original signal intensity method in the same set of specimens.

1.3.3 Specific Aim 3

Determine if T_2^ relaxation time can be used to predict semi-quantitative histological outcomes of the healing ACL.*

This aim was designed to test if the same MR variables (T_2^* and Volume) that could predict biomechanical performance in the healing ACL can also be used to predict histological outcomes for assessing ligament healing (Ligament Maturity Index). Using the same ligament specimens from Specific Aim 2, H&E stained sections for the ligaments were prepared and the slides were scored for a series of factors that are critical to ligament healing. Sub-scores based on the cell, collagen and vessel content were determined along with a cumulative score. We hypothesized that MR derived measures of T_2^* and volume would be significant predictors of the histological scoring of a healing ACL after 52 weeks of healing.

1.3.4 Specific Aim 4

Further refine and optimize the T_2^ relaxation time method from Specific Aim 2 & Specific Aim 3. Collect T_2^* data of the intact PCL using a 6 echo gold standard T_2^* method and two alternative methods for determining T_2^* and determine differences in fidelity and clinical feasibility between the methods.*

The purpose of this study was to compare two methods of T_2^* determination (2MM and 6LS_{ROI}) to a gold standard (6LS), to establish if the alternative methods could improve post-processing time without sacrificing fidelity of T_2^* values for eventual clinical translational. Using a 6 echo imaging sequence, two

alternative methods (2MM and 6LS_{ROI}) for determining posterior cruciate ligament (PCL) median T_2^* were compared to a gold standard method (6LS). We hypothesized that these two alternative approaches when used in tandem, would offer comparable T_2^* values and take significantly less post-processing time than the gold standard. If successful these two alternative approaches could be combined to offer a non-invasive tool for assessing ligament structural properties in a clinically feasible time frame.

1.3.5 Specific Aim 5

Determine intact ligament T_2^ relaxation time and volume for a population of human cadaveric knees of varying age, and determine the ability of the MR variables to predict ACL structural properties for a degenerating ligament.*

Our goal was to apply a T_2^* -based prediction model to a population of human cadaveric knees to determine if ligament degeneration could be predicted similar to ligament healing as presented in Specific Aim 1 & Specific Aim 2. A group of cadaveric knees was imaged *in situ* using the 6 echo T_2^* protocol from Specific Aim 4. The ACLs were then isolated and tensile tested to determine biomechanical performance. We hypothesized that MRI derived ligament volume and median ligament T_2^* would correlate to structural properties determined *ex vivo*.

1.3.6 Specific Aim 6

Assess the structural properties prediction model from Specific Aim 1 as it relates to clinical, functional and patient-oriented outcome measures from an existing clinical ACL reconstruction trial.

Using MR images from an ongoing ACL reconstruction clinical study, volume and SI models were created of patient grafts. Using this data and a statistical model based on Specific Aim 1, the MRI derived outcome measures were used to predict traditional outcome measures such as the hop test. We hypothesized that graft volume and signal intensity would correlate with measures of clinical, functional and patient outcome.

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Chapter 2

***In Situ* Non-Invasive T_2^* -Weighted MRI Derived Parameters Determine *Ex Vivo* Structural Properties of an ACL Reconstruction or Bio-enhanced Primary Repair in a Porcine Model**

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Abstract

Background: Magnetic resonance imaging (MRI) is a noninvasive technology that can quantitatively access ACL graft size and signal intensity. However, how those properties relate to reconstructed or repaired ligament strength during the healing process is yet unknown.

Purpose: We hypothesized that MR derived measures of graft volume and signal intensity are significant predictors of the structural properties of a healing ACL or ACL graft after 15 weeks and 52 weeks of healing.

Study Design: Controlled Laboratory Experiment Methods: The current data were gathered from two experiments evaluating ACL reconstruction and repair techniques. In the first experiment, pigs underwent unilateral ACL transection and received: 1) ACL reconstruction, 2) ACL reconstruction with collagen platelet composite (CPC), or 3) no treatment. The surgical legs were harvested following 15 weeks of healing. In the second experiment, pigs underwent ACL transection and received: 1) ACL reconstruction, 2) ACL reconstruction with CPC, 3) bio-enhanced ACL primary repair with CPC, or 4) no treatment. The surgical legs were harvested after 52 weeks. The harvested knees were imaged using a T_2^* weighted 3D-CISS sequence. Each ligament was segmented from the scans, and the intra-articular volume and the median grayscale values were determined. Mechanical testing was performed to establish the ligament structural properties.

Results: Volume significantly predicted the structural properties (maximum load, yield load, linear stiffness) of the ligaments and grafts ($R^2 = 0.56, 0.56, 0.49$; $p < 0.001$). Likewise, the median grayscale values significantly predicted the structural properties of the ligaments and grafts ($R^2 = 0.42, 0.37, 0.40$; $p < 0.001$). The combination of these two parameters in a multiple regression model improved the predictions ($R^2 = 0.73, 0.72, 0.68$; $p < 0.001$).

Conclusion: Volume and grayscale values from high resolution T_2^* weighted MRI images are predictive of structural properties of the healing ligament or graft in a porcine model.

Clinical Relevance: This study provides a critical step in the development of a non-invasive method to predict the structural properties of the healing ACL graft or repair. This technique may prove beneficial as a surrogate outcome measure in pre-clinical animal and clinical studies.

Keywords: MRI, ACL, Reconstruction, Structural Properties, Linear Regression

2.1 Introduction

Magnetic resonance (MR) imaging is widely used as a clinical tool for qualitatively monitoring anterior cruciate ligament (ACL) graft health following surgical reconstruction.[12] With the advent of stronger magnets and new imaging protocols, MR can now accurately measure the geometry of complex structures, such as the ACL. In addition, MR imaging can also provide information about the tissue quality by using different sequences to determine water content, fiber alignment and tissue density.[11] Therefore, in this study, we hypothesized that MR imaging also has the potential to provide a quantitative method for assessing the structural (failure) properties of an ACL graft or repaired ligament during healing, *in vivo*. A non-invasive MR method for predicting the structural properties of the graft or ligament would allow researchers to document functional healing within a subject in pre-clinical animal studies and clinical trials.

Determining the structural properties of the native ACL or reconstructed graft, *ex vivo*, is a common method for evaluating graft strength and documenting healing.[7,15,20] However, these methods require destructive testing and are not suitable for *in vivo* longitudinal assessment. Alternatively, signal intensity (also termed grayscale), a MR parameter that is a function of tissue type and water content, has been used to evaluate ACL graft integrity and maturation following ACL reconstruction surgery in humans.[3,8,13,16,18] The use of grayscale as an outcome measure is founded on research showing that the graft grayscale values decrease with time post-operatively,[18,20] and negatively correlates with its structural properties in an ovine model.[20] It has recently been established that graft volume when measured *in situ* via MR imaging, also correlates with the graft structural properties, and that the correlation could be improved by normalizing the volume to the graft T_2 relaxation time three months post-operatively in the caprine model.[5] While these findings are promising, the relationship between graft volume, grayscale value, and the graft structural properties throughout the healing process remains undocumented. Because bio-enhanced primary repair of the ACL is now proving to be efficacious in animal models,[19] it is also important to validate the relationships between graft volume, grayscale and the structural properties of repaired ligaments.

To date there have been no studies evaluating volume (a measure depicting the amount of tissue) and grayscale (a surrogate measure of tissue quality) as separate quantifiable MR variables to predict the structural properties of an ACL graft or ACL repair over the course of the healing process. The objective of this study was to assess a novel, non-invasive method for predicting the structural properties of a porcine ACL reconstructed ligament (graft) and a bio-enhanced ACL suture repair using 3D models derived from

T₂*-weighted MR imaging at two time points in the healing process. We hypothesize that intra-articular graft or ligament volumes and grayscale values will be significant predictors of the structural properties of the reconstructed ligament and ACL repairs after 15 weeks and 52 weeks of healing. Additionally, we hypothesize that the combination of volume and grayscale values, as two separate defining characteristics of the ligament, will improve that prediction in a multiple linear regression model.

2.2 Methods

2.2.1 Animal Model

Approval was obtained from the Institutional Animal Care and Use Committee (IACUC) prior to performing these studies.

2.2.1.1 15 Week Animals

Fifty adolescent Yucatan minipigs (approximately 15 weeks of age) underwent either ACL transection (10 animals), ACL transection immediately followed by ACL reconstruction with a patellar tendon allograft (10 animals), or ACL transection immediately followed by bio-enhanced ACL reconstruction using patellar tendon allograft and a collagen-platelet composite (30 animals) as previously described.[4] All surgical procedures were performed by the same orthopaedic surgeon. After 15 weeks of healing, the animals were euthanized and the surgical legs were harvested distal to the hip. MR imaging was performed before the joints were frozen and stored for mechanical testing.

2.2.1.2 52 Week Animals

As part of a different study utilizing the same animal model, a separate group of 32 Yucatan adolescent minipigs (approximately 15 weeks of age) underwent either ACL transection (8 animals), ACL transection immediately followed by ACL reconstruction with a patellar tendon allograft (8 animals), ACL transection immediately followed by bio-enhanced ACL reconstruction using patellar tendon allograft and a collagen-platelet composite (8 animals), or ACL transection immediately followed by bio-enhanced ACL repair with collagen-platelet composite (8 animals), as previously described.[4,10] All procedures were performed by

the same orthopaedic surgeon. After 52 week of healing, the knees were harvested, imaged, and stored for mechanical testing.

2.2.2 MR Imaging

A surface knee coil on a 3T MR scanner (Siemens TIM Trio, Erlangen, Germany) was used to image the joints. A T_2^* weighted 3D CISS sequence (Constructive Interference in the Steady State; TR/TE/FA, 12.9/6.5/ 35°; FOV, 160 mm; matrix 512X512, slice length/gap, 0.8mm/0; avg 1) was selected. This sequence produces high contrast between the soft tissues and joint fluid,[11,12] which optimizes the boundaries of the ligament or graft for manual segmentation from the image stack (Figure 2.1). For the 15 week animals, two scans from the ACL reconstruction group and six scans from the ACL reconstruction with collagen-platelet composite were omitted due to magnetic susceptibility artifact. For the 52 week animals, three scans from the ACL reconstruction group and two scans from the ACL reconstruction with collagen-platelet composite group were also omitted due to magnetic susceptibility artifact. It should also be noted that one transected animal in the 52 week time group was euthanized following surgery due to respiratory complications and was excluded.



Figure 2.1: 3D segmentation process illustrated on one sagittal slice of the image stack. A) 2D Graft location; B) Graft segmented; C) 3D model of graft.

2.2.3 3D Model and Volume Generation

Using commercially available software (Mimics 13.1, Materialise, Ann Arbor, Michigan), the reconstructed, repaired, and untreated ACL transected ligaments were segmented from the MR image stacks in both the coronal and sagittal planes (Figure 2.1). Three-dimensional (3D) surface models and grayscale volumes were created from the segmented images on a voxel by voxel basis. Intra-articular volumes and

median grayscale values were determined for the reconstructed ligaments, repaired ligaments, and untreated transected ligaments (note, that residual healing does occur in the transected ligaments in this animal model so segmentation is possible). The median grayscale values from the ligaments or grafts were normalized to the grayscale value of femoral cortical bone to account for any inter-scan variability.[2,17]

2.2.4 Structural Properties of the ACL/graft

An established tensile testing protocol was used to determine the structural properties of the reconstructed, repaired and transected ACLs.[4,14] The specimens were thawed to room temperature. The femur was transected just distal to the hip while the tibia was transected just proximal to the ankle to preserve the length of the long bones. The soft tissues were dissected from the tibia and femur while leaving the joint capsule intact. The proximal end of the femur and the distal end of the tibia were potted in 6 inch and 4 inch lengths of 1.5" PVC pipe, respectively, using a urethane resin (Smooth-On, Easton, Pennsylvania). All residual soft tissue and joint structures were then removed from the joint leaving only the femur-ligament-tibia complex intact. Using a servohydraulic material testing system (MTS 810; Prairie Eden, MN), the tensile loads were applied at 20mm/min to failure as previously reported.[14] Initially, the joint was placed at 30 degrees of flexion so that the mechanical axis of the ligament was collinear with the direction of pull of the tensile testing actuator. Starting with a tibiofemoral compressive force of 5 N, the entire load-displacement curve was recorded until a precipitous drop in load occurred. Yield load, maximum load, and linear stiffness values of the ligaments were calculated as previously described.[14]

2.2.5 Data Analysis

2.2.5.1 Relationship between MRI-derived parameters and structural properties

Because the grayscale values were not normally distributed, the log base 2 transforms of the grayscale values were used for all subsequent analyses. The reconstructed, repaired, and untreated transected ligaments at both 15 and 52 week time points were grouped together and analyzed as a single data set. First, linear regression models were used to separately test the relationships between: a) MR volume and structural properties and, b) median grayscale value and structural properties. Subsequently, both volume and median grayscale were included in a multiple linear regression model to predict the structural

properties (a fit plane). The R-square values for models were reported as indicators of the strength of the relationships since p-values alone may indicate a highly consistent or non-random relationship even in the presence of relatively poor prediction. The individual p-values of the covariates of the volume and median grayscale value in the regression were used to test the contribution of these variables to the regression and as a check against concerns over multicollinearity. As an additional check of the model fit, the predicted maximum loads across specimens were plotted against the actual experimental maximum loads to visualize the standard error of the regressions.

2.2.5.2 Volume and Grayscale differences between 15 and 52 week treatment groups

The ability of the volume and grayscale parameters to detect differences between the 15 week and 52 week treatment groups was tested using a one-way analysis of variance (ANOVA). Two ANOVAs were used, one using volume as the dependant variable and the other using the log base two of median grayscale value, comparing the 15 week and 52 week treatment groups.

2.2.6 Results

2.2.6.1 Relationship between MRI-derived parameters and structural properties

The volume of reconstructed, repaired, and untreated transected ligaments at both 15 and 52 week time points significantly predicted maximum failure load, yield load, and linear stiffness values; $R^2=0.56$, 0.56 , and 0.49 , respectively ($p<0.001$). The median grayscale value was also a significant predictor of maximum failure load, yield load, and linear stiffness, $R^2=0.42$, 0.37 , and 0.40 , respectively ($p<0.001$) (Figure 2.2). It should be noted that, during mechanical testing, all specimens failed mid-substance.

Using volume in conjunction with the median grayscale value, the multiple linear regression model predictions of maximum failure load, yield load, and linear stiffness for all specimens at both time points were improved; $R^2=0.73$, 0.72 and 0.68 , respectively ($p<0.001$) (Table 2.1). Both volume and median grayscale value significantly contributed to the regression equations (both $p<0.001$); with an increase in volume and/or a decrease in grayscale associated with higher structural properties of the reconstructed, repaired and transected ligaments (Figure 2.3). Comparing the actual value of structural properties to the predicted value of the structural properties using the multiple regression model; the standard error of the prediction plane estimate was 216.1N , 196.0N and 36.1N/mm for maximum load, yield load and linear stiffness, respectively (Table 1; Figure 2.4).

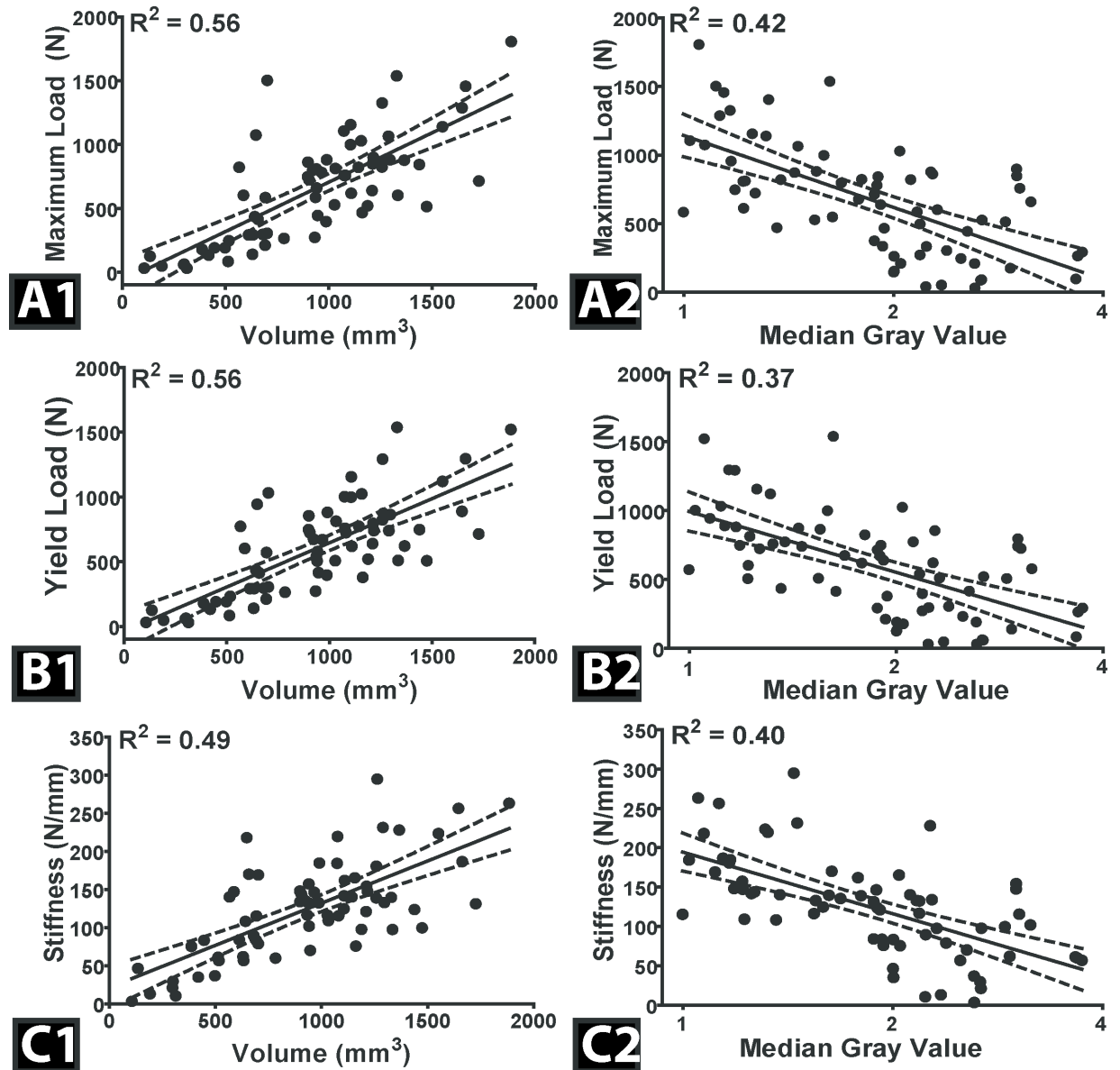


Figure 2.2: The graft structural properties for the 15 and 52 week specimens (A- Maximum Load, B- Yield Load, and C- Linear Stiffness) as a function of ligament volume (A1, B1 and C1) and the median grayscale value (A2, B2 and C2) in the linear regression models. Dashed lines represent 95% confidence interval.

2.2.6.2 MRI-derived parameter differences between 15 and 52 week treatment groups

The mean of the MRI-derived reconstructed, repaired and transected ligament volumes at 15 weeks and 52 weeks were $944 \pm 380.7 \text{ mm}^3$ and $908 \pm 435.2 \text{ mm}^3$ respectively, though the difference was not significant ($p=0.724$). The mean of the median grayscale values of the reconstructed, repaired and transected ligaments at 15 weeks and 52 weeks were 1.06 ± 0.48 and 0.61 ± 0.44 , respectively. The ligaments at 15 weeks had a significantly higher mean median grayscale value than ligaments at the 52 week time point ($p<0.001$; Figure 2.3).

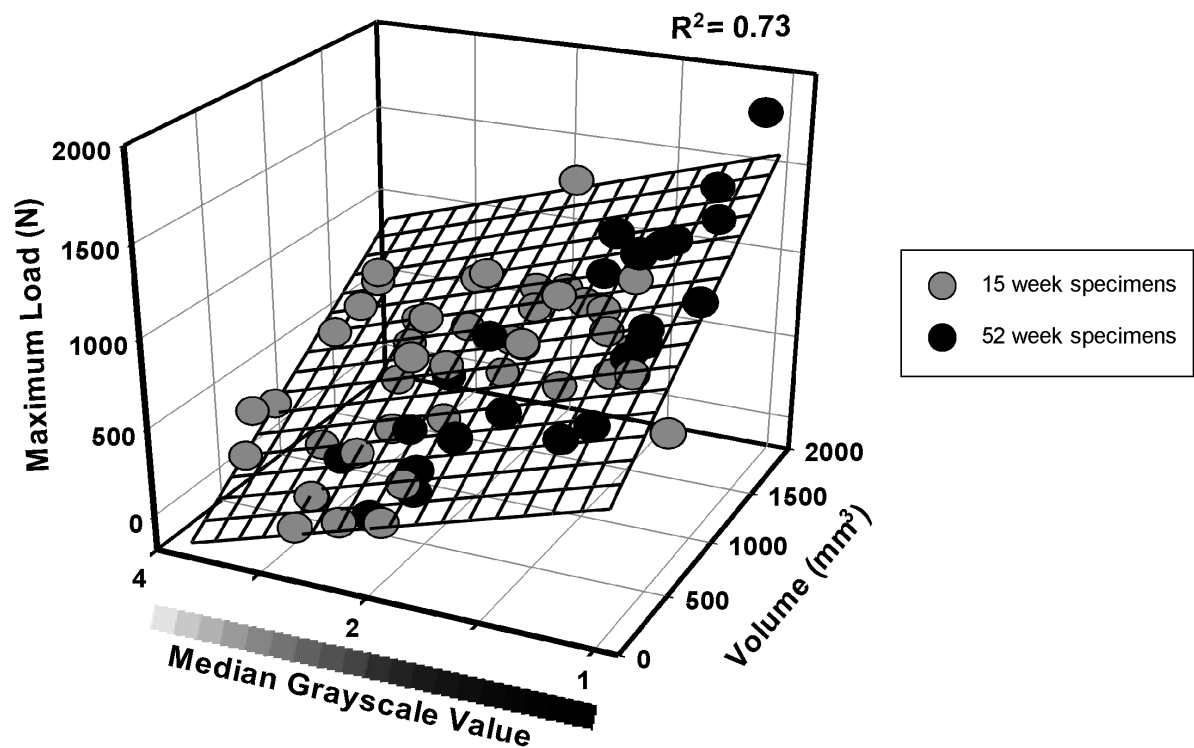


Figure 2.3: The reconstructed, repaired and transected ligament prediction plane for maximum load as a function of volume and of median grayscale value. The ligaments at 52 weeks (black circles) had a significantly lower median grayscale value than ligaments at the 15 week time point (gray circles) ($p<0.001$). Similar plots were found for the yield load and the linear stiffness prediction models.

2.2.7 Discussion

A quantitative method for accurately monitoring the post-operative healing of an ACL reconstruction or bio-enhanced primary ACL repair would be extremely valuable in both research and clinical settings. MRI is already widely available as a tool for non-invasive knee imaging and its ability to differentiate between

joint structures[11] and indirectly quantify graft maturation[20] makes it an ideal technology to evaluate ligament and graft healing. In our study, T_2^* weighted MRI derived volumes and median grayscale values were significantly related to the magnitude of maximum failure load, yield load and linear stiffness of reconstructed grafts, repaired and untreated transected ligaments. Expanding on this, we found by combining the volume and median grayscale values of the reconstructed, repaired and transected ligaments, the predictions for the structural properties were improved when compared to each variable alone. This prediction method maybe advantageous for evaluating results non-invasively at early time points, thus removing the need for euthanasia and mechanical testing at those time points and reducing the number of animals required for a study. Furthermore, these findings may have clinical implications for tracking post-operative changes with graft healing in patient cohort studies and randomized controlled trials.

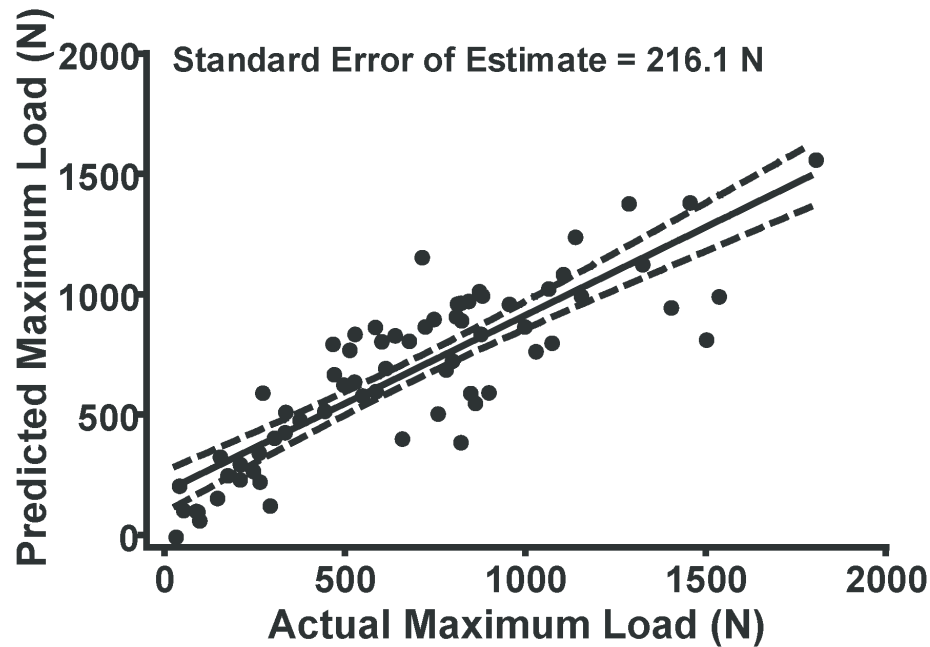


Figure 2.4: Actual maximum load versus predicted Maximum Load for both 15 and 52 week time points, calculated using volume and of median grayscale value.

Our findings align with a previous study which found that intra-articular graft volume, when normalized to the MR T_2 -relaxation time of the graft, increased the correlations to graft structural properties over volume alone, after 6 weeks of healing in the caprine model.[5] However, the prior study did not find a significant correlation between structural properties and T_2 values as a separate quantifiable variable. This could be explained by the single 6 week time point investigated with this study, which would have limited T_2 values to one phase of graft healing. By including an additional time point the current study

encompasses more than one phase of the healing response and the associated range of grayscale values. With this time point included a significant relationship was found between the grayscale parameter and structural properties alone as well as combined with volume in a multiple linear regression. Furthermore, in previous studies, quantitative MRI grayscale parameters[5,20] or clinician graded grayscale based scores[3,9,13,16,18] were determined using 2D mid substance MRI slices of a graft. The method presented herein analyzes the median grayscale value of the whole graft by reconstructing high resolution 3D images of the graft volume. This could minimize inaccuracies associated with consistently locating a “mid-substance” slice with a 2D method.

Traditionally, standard imaging techniques have been used to predict the strength of intact cadaveric ACLs.[1,6,7] These methods rely on photographic technology to generate 3D morphological models of the ACL. Results from these studies suggest that ACL volume could be used in a regression model to predict the structural properties of the native ACL.[7] These standard imaging techniques provide a valuable method for predicting the structural properties of an intact ligament and may also be useful for the evaluation of the integrity of ACL grafts or repairs. However, these techniques require destruction of the joint to expose the ACL and obtain measurements and as a result could not be used with *in vivo* clinical applications. The regression results of the non-invasive MR technique in our study support the human cadaveric findings of volume being predictive of ACL structural properties. We found that two non-invasively obtained quantifiable variables, volume and median grayscale values, could both be used in a multiple regression analysis to characterize structural properties of a reconstructed, repaired and transected ligament. The significant contribution of the volume and grayscale parameters in the multiple regression equations indicates that the role of both independent variables should be considered in a predictive model (Table 2.1). This was verified by the increased R^2 values seen in the multiple regression predictions of structural properties compared to the single predictor R^2 values ($p < 0.0001$). This signifies that the combination of volume and median grayscale value offers a more complete evaluation of graft integrity than either parameter alone. The relatively low standard errors of the predictions suggest that the measured volume and median grayscale values could be effective in future use of the regression prediction models following further validation.

In addition to the correlation between reconstructed ligament grayscale (signal intensity) and structural properties, a prior MR investigation also looked at signal-to-noise quotient (another MR parameter assessing grayscale) differences over time.[20] Histological results from the graft healing process were used to indirectly confirm the remodeling process in parallel with signal-to-noise quotient changes.[20] While the study conducted herein did not include histological evidence of the healing process, the grayscale

Dependent Variable (Y)	Predictor (X1, X2)	P-value	R ²	Standard Error	Equation
Max Load (N)	VOL (mm ³)	<0.001	0.73	216.1	Max Load = 440.1 + 0.6*VOL – 370.1*MGV
	MGV	<0.001			
Yield Load (N)	VOL (mm ³)	<0.001	0.72	196.0	Yield Load = 343.8 + 0.6*VOL – 298.5* MG V
	MGV	<0.001			
Stiffness (N/mm)	VOL (mm ³)	<0.001	0.68	36.1	Stiffness = 141.0 + 0.1*VOL – 37.5* MG V
	MGV	<0.001			

Table 2.1: Summary of the reconstructed ligament structural property prediction equations for both the 15 and 52 week time points as a function of volume (VOL) and median grayscale value (MGV).

differences observed between the 15 week and 52 week groups show a similar decreasing trend with time and could indicate healing and maturation of the graft or ligament on a tissue level. For the volume parameter, no difference was observed between the 15 week and 52 week groups.

There are study limitations that should be considered. First, the knees were imaged post-mortem. In a clinical situation, *in vivo* artifacts due to blood flow may affect the results. Nonetheless, the non-invasive MR method utilized in the present study, which is based on graft volume and grayscale, shows considerable promise for use *in vivo*. Second, the grayscale variable used to represent signal intensity can vary depending on MR imaging parameters. We normalized the grayscale values to that of cortical bone within each image to minimize this concern. For this analysis, we did not separate out the group effects. It is possible that the CPC could affect the prediction. However, this possibility seems unlikely given the high coefficients of determination and that the addition of CPC did not affect the slope of the prediction of the structural properties relative to the MR graft volume.[5] Finally, inherent differences between the reconstructed, repaired and transected ligament treatments could affect the relationship of the volume and grayscale parameters to structural properties. However, taking into account that the failure mechanism for all specimens was mid-substance and that the intra-articular portion of each ligament analyzed was consistent between the reconstructed, repaired or transected ligaments; it can be reasonably assumed that the MRI derived variables represented the same functional unit between specimens. Despite these limitations, we have shown that MRI derived morphology and grayscale can be used to predict structural properties of a reconstructed, repaired, and untreated transected ACL. We now plan to longitudinally validate this non-invasive MR based prediction method for documenting within-subject temporal changes relating to ACL strength and healing between treatment groups.

2.3 Conflicts of interest

None.

2.4 Acknowledgements

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Chapter 3

T₂* MR Relaxometry and Ligament Volume are Associated with the Structural Properties of the Healing ACL

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Abstract

Our objective was to develop a non-invasive magnetic resonance (MR) method to predict the structural properties of a healing anterior cruciate ligament (ACL) using volume and T_2^* relaxation time. We also compared our T_2^* -based structural property prediction model to a previous model utilizing signal intensity, an acquisition-dependent variable. Surgical ACL transection followed by no treatment (i.e., natural healing) or bio-enhanced ACL repair was performed in a porcine model. After 52 weeks of healing, high-resolution MR images of the ACL tissue were collected. From these images, ligament volumes and T_2^* maps were established. The structural properties of the ligaments were determined via tensile testing. Using the T_2^* histogram profile, each ligament voxel was binned based on its T_2^* value into four discrete tissue sub-volumes defined by specific T_2^* intervals. The linear combination of the ligament sub-volumes binned by T_2^* value significantly predicted maximum load, yield load, and linear stiffness ($R^2 = 0.92, 0.82, 0.88$; $p < 0.001$) and were similar to the previous signal intensity based method. In conclusion, the T_2^* technique offers a highly predictive methodology that is a first step towards the development of a method that can be used to assess ligament healing across scanners, studies, and institutions.

Keywords: *MRI, ligament healing, ACL, structural properties, biomechanics*

3.1 Introduction

Biomechanical measurements of the structural properties of the anterior cruciate ligament (ACL) are frequently used to document functional healing after surgical ACL repair and reconstruction in pre-clinical animal models.[1–5] Despite being a useful quantitative measure of graft healing,[1–3] the current techniques to quantify the structural properties require harvesting the joint and testing the ligament to failure. Therefore, these current methods are inherently unsuitable for *in vivo* longitudinal assessment in both animal studies and human clinical trials.

Alternatively, magnetic resonance (MR) imaging is a widely available, non-invasive tool that has the potential to predict the biomechanical properties of ACL treatments.[6] MR graft signal intensity has been found to correlate to the structural properties measured via *ex vivo* mechanical testing.[3] Building on these initial studies, we found that the combination of MR ligament volume (a measure of tissue quantity) and the median ligament signal intensity (a surrogate measure of tissue quality) within that volume can be incorporated into a first order multiple regression model to improve the accuracy of the prediction of the structural properties.[7] This new technique offered a more complete evaluation of graft integrity than either volume or signal intensity alone.[7]

However, the use of signal intensity as an outcome measure is limited by its dependence on image acquisition parameters and scanner manufacturer, rendering the predictions to be protocol, magnet, and hence, institution specific. One way to standardize MR results between scanners is to use relaxation time variables, such as T_2 and T_2^* . These variables are inherent tissue properties that reflect specific tissue characteristics, and are much less sensitive to image acquisition parameters than conventional signal intensity data.[8] T_2^* relaxation time is a MR parameter that has been shown to correlate with the level of tissue organization, and is thus well suited for imaging highly organized collagenous structures,[9–12] such as ligaments and tendons. Thus, T_2^* relaxation time could provide a more universal prediction model of the structural properties of a healing ligament that would be applicable across scanners of the same strength and between institutions.

The purpose of this study was to establish the relationship between ligament volume, T_2^* relaxation time, and the structural properties of a healing ligament in a porcine model of ACL repair. We hypothesized that a multiple regression model based on ligament volume and its corresponding T_2^* values would provide a noninvasive predictor of the ligament's structural properties after 52 weeks of healing. As a secondary aim, we compared the proposed T_2^* -based structural properties prediction model to our previous model that

incorporates signal intensity instead of T_2^* . [7] We hypothesized that the coefficients of determination would be greater and that the standard errors would be less when using the T_2^* prediction method compared to the signal intensity method.

3.2 Methods

3.2.1 Animal Model

Approval was obtained from the Institutional Animal Care and Use Committee prior to performing these studies. Fifteen adolescent Yucatan minipigs (approximately 15 weeks of age) underwent unilateral ACL transection surgery as previously described. [13,14] Immediately following transection, eight of the animals received bio-enhanced ACL repair with an extracellular matrix-blood composite (Bio-Enhanced Repair, or BE-ACL group) and seven were left untreated to heal naturally without repair (ACL transection, or ACLT group). [13] All animals made it to 52 weeks with no complications, at which point all fifteen operative knees were harvested and immediately imaged. Following imaging, the specimens were frozen and stored at -20 degrees Celsius until mechanical testing.

3.2.2 MR Imaging

A surface knee coil on a 3T MR scanner (TIM Trio; Siemens, Erlangen, Germany) was used to image the joints. Two separate imaging protocols were performed on each knee: 1) a dual echo protocol to determine T_2^* relaxation time, and 2) a manufacturer provided protocol to determine signal intensity. [7] The images used for the signal intensity determination and analysis were a subset of those used in another study investigating the relationship between volume, signal intensity, and ligament structural properties over the course of healing. [7] There was no intra-articular artifact found in any of the specimens. The BE-ACL group had a titanium button for suture fixation on the anterolateral cortical bone of the femur but was sufficiently far from the intra-articular space to avoid issues with artifact with the ACL.

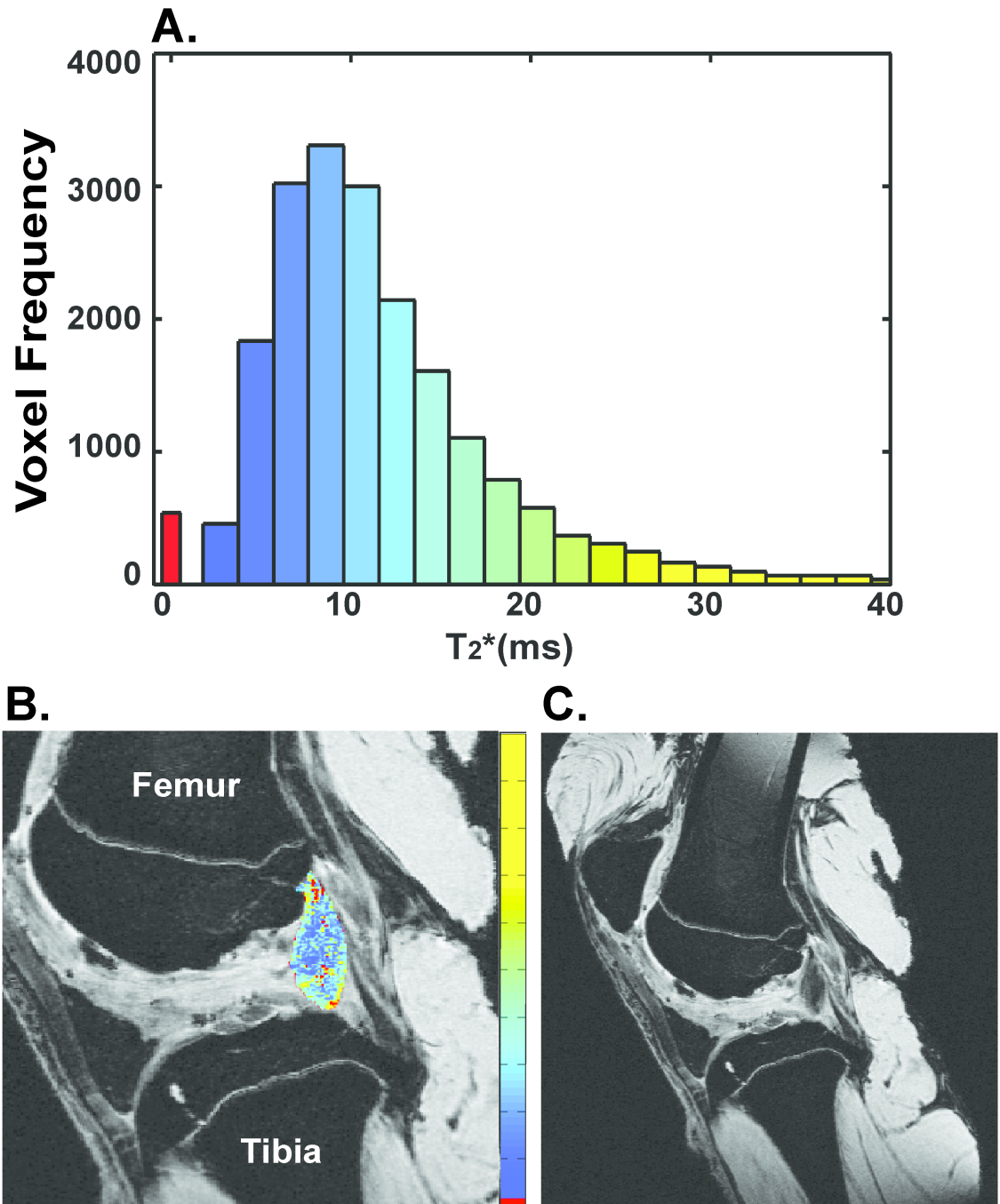


Figure 3.1: Example ligament histogram showing (A) the bimodal distribution for T_2^* with associated T_2^* first quartile (Q1), median (Q2) and third quartile (Q3) summary statistics, (B) the T_2^* ligament map, and (C) the original DICOM image. Note the ligament voxels illustrated in a red (B) represent voxels with a T_2^* value of 0 ms. The MR images are a sagittal view of the femoral notch with the femur at the top of the image and the tibia at the bottom. For the MR images shown $TE = 7.36$ ms.

3.2.2.1 MR Imaging: T_2^* determination

To determine T_2^* , a high-resolution T_1 -weighted gradient echo 3-D FLASH dataset (note: T_1 weighted images are used to derive T_2^* relaxation time) utilizing two echo times (TR/TE/FA, 25/7.36 & 15.24/12°; FOV, 140 mm; matrix 512X512; slice length/gap, 0.85mm/0; avg, 3; bandwidth, 130; scan time 19 minutes) was acquired of the injured knee immediately after harvest. High-resolution 3-D image acquisition was required to optimally capture the relatively small structure of the healing ACL. The healing ligaments and associated peri-ligamentous scar tissue were then manually segmented from these T_1 -weighted MR images using commercially available software (Mimics 14.1; Materialize, Ann Arbor, MI). 3-D models of the healing ligaments were created using previously described methods.[7] Summing the total number of ACL voxels provides an estimate of the whole ligament volume (16.1 voxels equaled one mm³). Using custom Matlab (R2012b; MathWorks, Natick, MA) code, T_2^* maps were calculated using the following signal intensity (SI) relationship.[15]

$$T_2^* = \left(\frac{\ln SI_1 - \ln SI_2}{TE_2 - TE_1} \right)^{-1} \quad (3.1)$$

SI_1 and SI_2 are the signal intensities corresponding to the echo times TE_1 and TE_2 where $TE_2 > TE_1$ for each voxel (note: TR was the same for both echo times allowing for the determination of T_2^* using a two echo fit). To ensure the relaxation time maps used in this study were T_2^* weighted, the images used to create the maps were gradient echo acquisitions, and the echo times were significant compared to the T_2^* distribution of the tissue under examination.[9]

To produce ligament specific maps, the voxels corresponding to the ligament were extracted from the T_2^* maps using the 3-D models created from the segmented images (Figure 3.1). Histograms of the voxel-wise T_2^* values were plotted using these ligament specific maps. Two distinct peaks of relaxation times were apparent with no overlap within each healing ligament (Figure 3.1). Further, the voxels making up these peaks were spatially organized such that the first voxel peak represented those with $T_2^*=0$ ms and was generally located within the central portion of the ligament. Presumably, the voxels with $T_2^*=0$ ms have a range of short T_2^* values below our MR protocol's measurable limit (4.8 ms, the theoretical limit based on voxel signal to noise ratio)[16], and would therefore fall between 0 and 4.8 ms. The second peak formed a lognormal distribution of relaxation times, where voxels with lower T_2^*

values were primarily found in the central portion of the ligament while higher T_2^* values were identified towards the periphery (Figure 3.1). The whole ligament volume was then binned into four separate tissue sub-volumes (Vol_1 , Vol_2 , Vol_3 , Vol_4) with equal T_2^* intervals up to 50 ms (0-12.5; 12.6-25; 25.1-37.5; 37.6-50 ms, respectively) (Figure 3.2). Tissue volume in terms of mm^3 (note: for this MR protocol 16.1 voxels equaled one mm^3) was calculated for each sub-volume.

3.2.2.2 MR Imaging: Signal intensity determination

During the same imaging session an additional single set of MR images was acquired to determine signal intensity. To accomplish this a T_2^* weighted 3-D CISS sequence (note: signal intensity was derived from a single set of T_2^* weighted images) (TR/TE/FA, 12.9/6.5/ 35°; FOV, 140 mm; matrix 512x512, slice length/gap, 0.8mm/0; avg 1) was performed to establish the median signal intensity and volume of the whole ligament using our previously established multiple regression model.[7] The healing ligaments were segmented from these MR images and 3-D models of the healing ligaments were created. From the models, the whole ligament volume (VW_{SI}) in terms of mm^3 (note: for this MR protocol 17.1 voxels equaled one mm^3) was determined. Histograms of signal intensity in terms of grayscale values normalized to the signal of posterior femoral cortical bone (normalization standard) were plotted.[9,17] Histograms of signal intensity were found to have a single uniform distribution for each ligament (Figure 3.3). Signal intensity in terms of median gray scale value (MGV_{SI}) was calculated from each distribution for the whole ligament.

3.2.3 Structural Properties of the healing ACL

An established tensile testing protocol was used to determine the structural properties of the repaired and untreated transected ACLs after 52 weeks of healing.[18,19] The specimens were thawed to room temperature. The proximal end of the femur and the distal end of the tibia were potted in PVC pipe using a urethane resin. The joint was carefully dissected, leaving only the femur-ligament-tibia complex intact and all associated peri-ligamentous scar tissue. Using a servohydraulic material testing system (MTS 810; Prairie Eden, MN), the tensile loads were applied at 20 mm/min to failure as previously reported.[18] Initially, the joint was placed so that the mechanical axis of the ligament was collinear with the direction of pull of the actuator. Starting with a tibiofemoral compressive pre-load of 5 N, the entire tensile load-displacement curve was recorded until a precipitous drop in load occurred. The maximum

load, yield load, and linear stiffness values of the ligaments were calculated from the load-displacement data.[18]

3.2.4 Data Analysis

First order multiple linear regression analyses (SigmaPlot 12.0; Systat Software Inc., San Jose, CA) were used to find the best-fit parameters and test the relationship between each ligament's sub-volume and the respective structural properties in the T_2^* model. The resulting model included a volume term (Vol_1 , Vol_2 , Vol_3 , Vol_4) representing each of the four bins. Each bin was defined by its associated interval of T_2^* (0-12.5; 12.6-25; 25.1-37.5; 37.6-50 ms, respectively). Note that the voxels with a T_2^* of 0 ms were included in Vol_1 because their relaxation times fall into the 0-12.5 ms bin range. The R-square values were reported as indicators of the relationship strength and goodness of fit. The p-values of the covariates (Vol_1 , Vol_2 , Vol_3 , Vol_4) in the regression tested the contribution of the T_2^* defined sub-volumes to the model. The predicted structural properties (maximum load, yield load, and linear stiffness) across specimens were plotted against the actual experimental structural properties to visualize the standard error of the regressions as an additional check of the T_2^* model fit.

Additionally the volume of the whole ligament (VW_{SI}) and signal intensity (MGV_{SI}) values from each ligament were used in a first order multiple linear regression analysis to predict the structural properties as previously described.[7] The predictions of the T_2^* model and the signal intensity model were compared using the mean R-square values and their respective 95% confidence limits (CL).[20] Percent overlap of the confidence limits was tested using a z test statistic ($\alpha=0.05$) to evaluate the differences between models.[21,22]

3.3 Results

3.3.1 T_2^* Model Prediction

Of the T_2^* derived parameters evaluated, the best prediction of mechanical properties was a linear combination of the ligament sub-volumes (Vol_1 , Vol_2 , Vol_3 , Vol_4) defined by their respective intervals of T_2^* values (Table 3.1). The R-squared values of the T_2^* prediction equation for maximum load, yield load and linear stiffness were 0.93, 0.78, and 0.88, respectively ($p<0.001$ for all). The 95% confidence

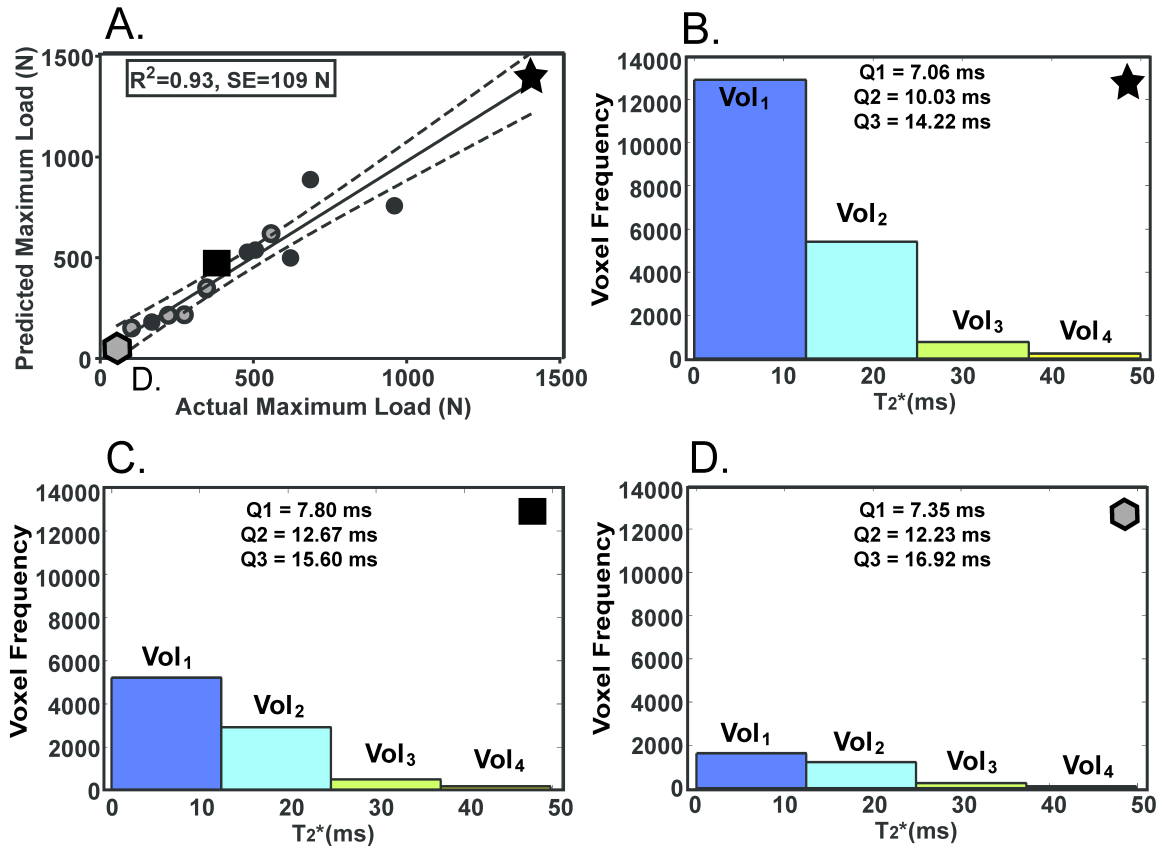


Figure 3.2: T_2^* model: (A) Actual versus predicted maximum load calculated using the linear combination of Vol₁, Vol₂, Vol₃ and Vol₄. The dotted lines represent the 95% confidence intervals. Gray shapes represent transected ligaments while black shapes represent repaired ligaments. The highest (star, B), median (square, C) and lowest (hexagon, D) maximum load ligaments and their corresponding histogram profile are also represented with associated T_2^* first quartile (Q1), median (Q2) and third quartile (Q3) summary statistics.

limits for these R-squared values of maximum load, yield load and linear stiffness were [0.92, 0.94]; [0.75, 0.81]; [0.86, 0.90], respectively. Standard errors for the prediction of maximum load (Figure 3.2), yield load (Figure 3.3A), and linear stiffness (Figure 3.3B) were 109 N, 141 N and 26 N/mm respectively (Table 3.1).

Dependent Variable (Y)	Predictor Term	T ₂ * Range (ms)	p	R ²	R ² 95% CL	Standard Error	Predictor Term Contribution per unit volume (mm ³)	Equation
Max Load (N)	Vol ₁	0 - 12.5	<0.001	0.93	[0.92, 0.94]	109 N	1.55 N	Max Load = -161.5 + (1.55 × Vol ₁) + (1.20 × Vol ₂) - (1.62 × Vol ₃) - (5.48 × Vol ₄)
	Vol ₂	12.6 - 25	0.396				1.20 N	
	Vol ₃	25.1 - 37.5	0.891				-1.62 N	
	Vol ₄	37.6 - 50	0.746				-5.48 N	
Yield Load (N)	Vol ₁	0 - 12.5	0.005	0.78	[0.75, 0.81]	141 N	1.13 N	Yield Load = 55.3 + (1.13 × Vol ₁) + (0.04 × Vol ₂) - (1.24 × Vol ₃) - (1.64 × Vol ₄)
	Vol ₂	12.6 - 25	0.983				0.04 N	
	Vol ₃	25.1 - 37.5	0.936				-1.24 N	
	Vol ₄	37.6 - 50	0.940				-1.64 N	
Stiffness (N/mm)	Vol ₁	0 - 12.5	<0.001	0.88	[0.86, 0.90]	26 N/mm	0.28 N/mm	Stiffness = -1.8 + (0.28 × Vol ₁) + (0.02 × Vol ₂) + (0.55 × Vol ₃) - (0.70 × Vol ₄)
	Vol ₂	12.6 - 25	0.961				0.02 N/mm	
	Vol ₃	25.1 - 37.5	0.845				0.55 N/mm	
	Vol ₄	37.6 - 50	0.860				-0.70 N/mm	

Table 3.1: T₂* model: Summary of ligament structural property prediction equations as a function of ligament sub-volumes (Vol₁, Vol₂, Vol₃, Vol₄) defined by range of T₂* values.

3.3.2 Signal Intensity Model Prediction

Of the signal intensity derived parameters studied, the best predictors of mechanical properties were a linear combination of the whole ligament volume (VW_{SI}) and the signal intensity (MGV_{SI}) (Table 3.2). The R-squared values of the signal intensity prediction equations for maximum load, yield load, and linear stiffness were 0.84, 0.92, and 0.88 respectively (p<0.001 for all) (Figure 3.4). The 95% confidence limits for these R-squared values of maximum load, yield load and linear stiffness were [0.80, 0.88]; [0.90, 0.94]; [0.85, 0.91], respectively. Standard errors for the prediction of maximum load, yield load, and linear stiffness were 155 N, 75 N and 24 N/mm respectively (Table 3.2).

There was no overlap of the R-squared confidence limits between the T₂* and signal intensity models for the maximum load prediction. For maximum load the T₂* model displayed significantly higher R-squared confidence limits than the signal intensity model. There was also no overlap of R-squared confidence limits between the T₂* and signal intensity models for the yield load prediction. In this case the signal intensity model displayed significantly higher R-squared confidence limits than the T₂* model. There was

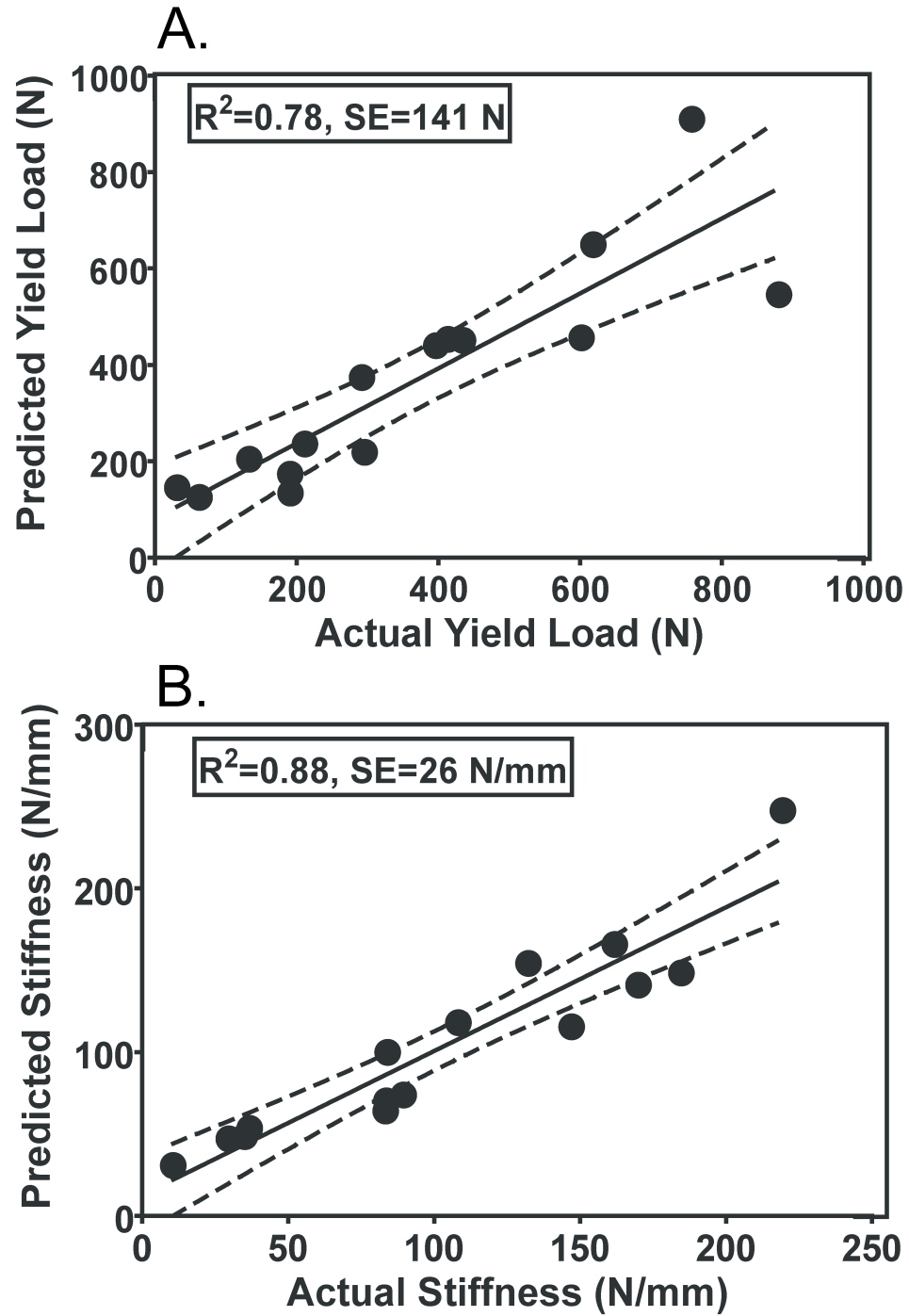


Figure 3.3: T_2^* model: (A) Actual versus predicted yield load (B) and actual versus predicted linear stiffness plots calculated using the linear combination of Vol₁, Vol₂, Vol₃ and Vol₄. The dotted lines represent the 95% confidence intervals.

Dependent Variable (Y)	Predictor Term	p	R ²	R ² 95% CL	Standard Error	Equation
Max Load (N)	VW _{SI}	<0.001	0.84	[0.80, 0.88]	155 N	Max Load = 299.8 + (0.8 × VW _{SI}) - (424.7 × MGVS _{SI})
	MGVS _{SI}	0.006				
Yield Load (N)	VW _{SI}	<0.001	0.92	[0.90, 0.94]	75 N	Yield Load = 340.2 + (0.5 × VW _{SI}) - (372.4 × MGVS _{SI})
	MGVS _{SI}	<0.001				
Stiffness (N/mm)	VW _{SI}	<0.001	0.88	[0.85, 0.91]	24 N/mm	Stiffness = 73.6 + (0.1 × VW _{SI}) - (75.9 × MGVS _{SI})
	MGVS _{SI}	0.002				

Table 3.2: Signal Intensity model: Summary of ligament structural property prediction equations as a function of ligament whole volume (VW_{SI}) and signal intensity (MGVS_{SI}).

a 100% overlap of R-squared confidence limits for linear stiffness with the T₂* prediction interval nested within the signal intensity model interval making them statistically equivalent (Table 3.1 & 3.2).

3.4 Discussion

A non-invasive tool that can predict the biomechanical properties of a healing ligament would be highly valuable in a research and clinical setting for evaluating outcomes of different ACL treatments. Our objective was to develop a magnetic resonance (MR) method to predict structural properties of a healing anterior cruciate ligament (ACL) using volume and T₂* relaxation time. We found the linear combination of the ligament sub-volumes defined by increasing T₂* intervals (Vol₁, Vol₂, Vol₃, Vol₄) significantly predicted structural properties of a healing porcine ACL at 52 weeks post-operatively.

There are two parameters that contribute to the structural properties of a ligament; 1) the amount of tissue (as represented by the volume) and 2) the quality of the tissue (as represented by T₂*). Using a T₂* histogram profile, each ligament was partitioned into four tissue sub-volumes with equal intervals of increasing T₂*. In our regression model, we determined the relative proportions of the total ligament volume made up of high quality tissue (Vol₁) down to the lowest quality tissue (Vol₄) to determine how these proportions contribute to the overall ligament structural properties. The amount of tissue with a T₂* value between 0-12.5 ms (Vol₁) was found to be the most significant in predicting structural properties (p<0.001; Table 3.1). This would be expected as highly organized tissue has been associated with short T₂* values.[10] Contribution of this sub-volume to structural properties can be observed with its associated slope in the prediction equations (Table 3.1). Per unit volume of Vol₁ (T₂* values 0-12.5 ms), 1.55 N, 1.13 N, and 0.28 N/mm are contributed to maximum load, yield load and linear stiffness,

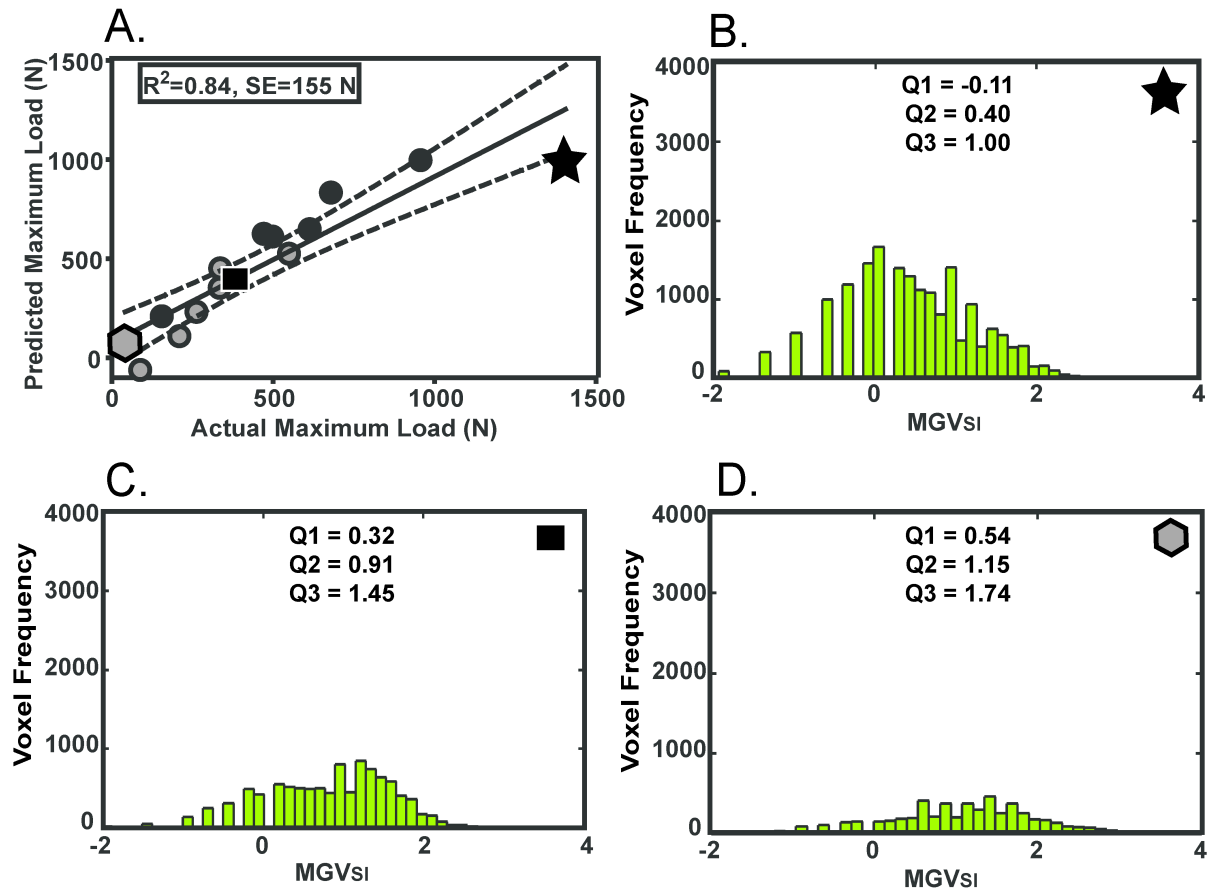


Figure 3.4: Signal intensity model: (A) Actual versus predicted maximum load calculated using linear combination of VW_{SI} and MGV_{SI} . The dotted lines represent the 95% confidence intervals. Gray shapes represent transected ligaments while black shapes represent repaired ligaments. The highest (star, B), median (square, C) and lowest (hexagon, D) maximum load ligaments and their corresponding histogram profile are also represented with associated SI first quartile ($Q1$), median (MGV_{SI} , $Q2$) and third quartile ($Q3$) summary statistics.

respectively. Generally less organized tissue in the T_2^* range of 12.6-25 ms (Vol_2) contributed less to structural properties per unit volume with 1.20 N, 0.04 N and 0.02 N/mm for maximum load, yield load and linear stiffness respectively. While Vol_2 , Vol_3 and Vol_4 were not found to be significant predictors in this model ($p>0.3$) they were kept as terms in the model to accommodate a broader range of T_2^* values that would be expected with phases of healing earlier (<52 weeks) and to include the whole ligament volume in the analysis. The composition of the voxels in Vol_3 and Vol_4 with higher ranges of T_2^* values is likely a combination of less densely packed collagen fibrils and periligamentous tissue of scar formation.

Additional analyses were performed to determine the effect of partitioning the ligaments into more sub-volumes (bins = 8) with smaller ranges of T_2^* intervals defining each sub-volume (see Supplement Table 3.3). The four-bin model was the simplest linear regression model that yielded relatively high R-squared and low standard errors for determining structural properties when compared to other bin numbers.

The distribution of T_2^* values for each ligament was found to be bimodal and exhibited two discrete peaks. The voxels in this first peak displayed a single T_2^* value of 0 ms. Presumably there is variability in this tissue type, however the relaxation times approaching zero were too short to be acquired using our current MR protocol. The impact of this first peak is relatively small, however, as the median percentage of ACL voxels with $T_2^*=0$ to the total number of voxels for the whole ACL was only 3.8% (1st Quartile = 2.5%; 3rd Quartile = 8.3%). Nonetheless, we know that the zero T_2^* values fall within the range of 0 and 4.8 ms. Despite not being able to capture the exact T_2^* value of the voxels under the theoretical 4.8 ms limit,[16] the current approach of partitioning or binning each ligament into four sub-volumes based on the T_2^* ranges helps to reduce the effects of minor observation error and allows these voxels to be accounted for in the prediction model. Unlike previous studies using a single average value for volume, signal intensity, or T_2 :[7,23] quantifying sub-volumes (Vol_1 , Vol_2 , Vol_3 , Vol_4) of tissue (with different levels of organization) based on their associated T_2^* values offers a more detailed evaluation of tissue composition and its relationship to structural properties. Models that neglect the heterogeneous composition of the healing ACL tissues do not account for the fact that these tissues likely have different material properties. Additionally, the histograms of individual ligaments (Figure 3.2) can be observed for the contribution of the sub-volumes (Vol_1 , Vol_2 , Vol_3 , Vol_4) to the structural properties prediction. The ligaments with the highest structural properties displayed larger sub-volumes of organized tissue represented by low T_2^* times (Vol_1), while ligaments with the lowest structural properties displayed smaller sub-volumes of organized tissue (Figure 3.2).

The T_2^* model was found to be comparable to the original signal intensity prediction model. While the

Dependent Variable (Y)	Predictor Term	T ₂ * Range (ms)	p	R ²	R ² 95% CL	Standard Error	Predictor Term Contribution per unit volume (mm ³)	Equation
Max Load (N)	Vol ₁	0-6.25	0.304	0.95	[0.946, 0.953]	122	1.76	$\text{Max (L)} = -146.79 + (1.76 \times \text{Vol}_1) + (2.04 \times \text{Vol}_2) - (2.36 \times \text{Vol}_3) + (13.26 \times \text{Vol}_4) - (16.26 \times \text{Vol}_5) - (0.64 \times \text{Vol}_6) - (16.94 \times \text{Vol}_7) + (17.11 \times \text{Vol}_8)$
	Vol ₂	6.3-12.5	0.339				2.05	
	Vol ₃	12.6-18.75	0.766				-2.36	
	Vol ₄	18.8-25	0.48				13.26	
	Vol ₅	25.1-31.25	0.621				-16.26	
	Vol ₆	31.3-37.5	0.988				-0.64	
	Vol ₇	37.6-43.75	0.719				-16.94	
	Vol ₈	43.8-50	0.697				17.11	
Yield Load (N)	Vol ₁	0-6.25	0.14	0.86	[0.851, 0.868]	146	3.17	$\text{Yield L (N)} = -116.88 + (3.17 \times \text{Vol}_1) - (0.81 \times \text{Vol}_2) + (4.87 \times \text{Vol}_3) + (4.47 \times \text{Vol}_4) - (41.45 \times \text{Vol}_5) + (49.04 \times \text{Vol}_6) - (18.75 \times \text{Vol}_7) - (10.16 \times \text{Vol}_8)$
	Vol ₂	6.3-12.5	0.742				-0.81	
	Vol ₃	12.6-18.75	0.608				4.87	
	Vol ₄	18.8-25	0.838				4.47	
	Vol ₅	25.1-31.25	0.306				-41.45	
	Vol ₆	31.3-37.5	0.352				49.04	
	Vol ₇	37.6-43.75	0.737				-18.75	
	Vol ₈	43.8-50	0.845				-10.16	
Stiffness (N/mm)	Vol ₁	0-6.25	0.492	0.93	[0.925, 0.934]	26	0.24	$\text{Stiffness} = 4.00 + (0.24 \times \text{Vol}_1) + (0.42 \times \text{Vol}_2) - (0.74 \times \text{Vol}_3) + (4.33 \times \text{Vol}_4) - (11.33 \times \text{Vol}_5) + (11.54 \times \text{Vol}_6) + (1.10 \times \text{Vol}_7) - (4.95 \times \text{Vol}_8)$
	Vol ₂	6.3-12.5	0.357				0.42	
	Vol ₃	12.6-18.75	0.66				-0.74	
	Vol ₄	18.8-25	0.29				4.33	
	Vol ₅	25.1-31.25	0.137				-11.33	
	Vol ₆	31.3-37.5	0.231				11.54	
	Vol ₇	37.6-43.75	0.911				1.10	
	Vol ₈	43.8-50	0.597				-4.95	

Table 3.3: Supplement 1: T₂* 8 bin model: Summary of ligament structural property prediction equations as a function of ligament sub-volumes (Vol₁, Vol₂, Vol₃, Vol₄, Vol₅, Vol₆, Vol₇, Vol₈) defined by range of T₂* values. First order multiple linear regression analyses (SigmaPlot 12.0; Systat Software Inc., San Jose, CA) were used to test the relationship between each ligament's sub-volume and respective structural properties. The R-square values were reported as indicators of the relationship strength and goodness of fit. None of the sub-volumes in the 8 bin analysis were found to be significant individual contributors (p>0.3) to the model, indicating the sub-volume T₂* intervals were too small to capture the specific effect of the tissue volume or T₂* values on structural properties. In comparison to the eight bin model, the four bin model yielded similar high R-squared values and low standard errors for determining structural properties but offered a simpler linear regression model.

T_2^* model did not display higher R-squared values for all structural properties as hypothesized, the 100% overlap seen in linear stiffness confidence limits between the T_2^* and signal intensity methods, along with the marginal differences in maximum load and yield load confidence limits, suggest that these two prediction models offer similar certainty when determining structural properties.

However, the T_2^* prediction model offers the benefit of using the T_2^* variable, which is an inherent tissue property and much less sensitive to image acquisition parameters than conventional signal intensity data. T_2^* is influenced by susceptibility gradients at a microscopic level (reflecting the quantity and distribution of free water) where signal intensity is influenced by sequence parameters and hardware effects that can vary between scanners and even between scan sessions.[8] Furthermore, the SI values must be normalized to a standard in the field of view, in this case posterior femoral cortical bone. With the SI method, finding an easy to identify and repeatable structure in the field of view, which is not potentially influenced by treatment effects, is difficult. Conversely, T_2^* is an inherent tissue property so a normalization standard is unnecessary, eliminating any associated confounding effects. Thus, T_2^* , with additional validation, may provide a more consistent assessment of the healing ligament across scanners of the same magnet strength than examination of signal intensity and could make the T_2^* predictions for scans performed across scanning intervals, studies, and institutions readily comparable.

Our findings are supported by previous research showing that volume normalized to a different relaxation time parameter (T_2) correlates to ACL reconstruction graft structural properties.[23] However, this previous study was limited by a non-significant correlation between structural properties and T_2 values as an independent quantifiable variable. The lack of correlation found between T_2 and structural properties was likely the result of relatively long echo times ($>10\text{ms}$) used to collect T_2 and may have been too long for gathering the short relaxation times of graft tissue.[24] Building on these earlier findings, the current study found that by utilizing T_2^* , we were able to better capture the shorter relaxation times that are inherent to organized structures such as ligaments and tendons.[10] Furthermore, we were able to identify a significant individual contribution of T_2^* in terms of ligament sub-volume defined by ranges of T_2^* values (see Supplement Figure 3.5). Additionally, a study using Ultra-short echo time (UTE) imaging found a correlation between level of collagen organization in the meniscus and T_2^* . Short T_2^* was correlated with more densely packed collagen fibers and longer T_2^* values were associated with less densely packed collagen and meniscal damage.[10] While our study did not utilize UTE imaging, tissues with shorter T_2^* values were linked to higher ligament structural properties. This suggests that T_2^* can serve as a proxy for tissue organization and remodeling can be identified with the MR methodology presented herein.

Dependent Variable (Y)	Predictor Term	p	R ²	R ² 95% CL	Standard Error	Equation
Max Load (N)	MedianT ₂ [*]	0.014	0.39	[0.31, 0.47]	289	Max Load = 1620.3 - (97.2 × MedianT ₂ [*])
Yield Load (N)	MedianT ₂ [*]	0.002	0.53	[0.45, 0.61]	180	Yield Load = 1333.8 - (81.3 × MedianT ₂ [*])
Stiffness (N/mm)	MedianT ₂ [*]	0.006	0.45	[0.37, 0.53]	48	Stiffness = 326.2 - (18.6 × MedianT ₂ [*])

Table 3.4: Supplement 2: Whole ligament Median T₂^{*} correlation to structural properties. To test the contribution of each ligament's T₂^{*} values without the influence of volume, the T₂^{*} values of the whole ligament were used to calculate the median relaxation time (Median T₂^{*}). First order linear regression analyses were used to test the relationship between each ligament's Median T₂^{*} and respective structural properties. The R-square and p-values were reported as indicators of the relationship strength. In comparison to the 4 bin analysis, which considers each ligament's sub-volume defined by T₂^{*} value, the R-squared values using only Median T₂^{*} value were lower and standard errors higher. This finding indicates, as found with previous signal intensity studies,[7] that the four bin technique which considers both ligament volume and T₂^{*} value offers a more complete evaluation of graft structural properties than either property alone.

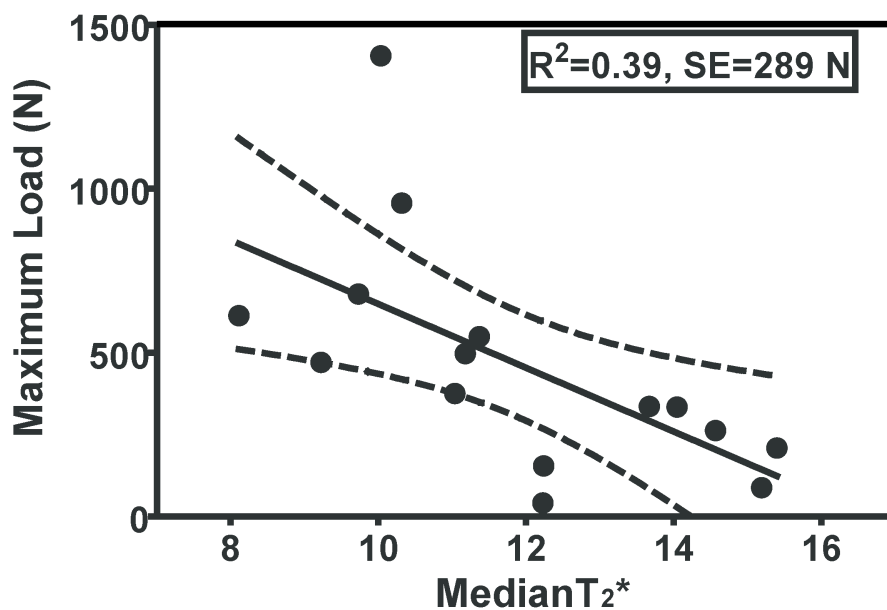


Figure 3.5: Supplement 3: Median ligament T₂^{*} versus actual maximum load. The dotted lines represent the 95% confidence intervals.

This study was limited by the use of only two echo times which adds uncertainty to the determination of relaxation time.[8] A two-point determination can overestimate relaxation time and is more sensitive to SI variability.[8,25] However, the correlation analysis between the median T_2^* and structural properties incorporates the inter-specimen variability in T_2^* . Additionally, this approach served to limit scan time (19 min), an important factor when developing a technique for clinical use. The standard errors for the structural properties predictions are comparable to the previous methods using signal intensity,[7] indicating an effective determination of T_2^* (Figure 3.2). A two point determination of T_2 has also been used in cartilage imaging[15,26] to limit scan time and maximize resolution when considering geometry was paramount, similar to the goal of our study. Further refinement of our imaging protocol could be achieved by collecting additional shorter echo times, which would allow for improved certainty in the T_2^* estimation using a nonlinear least-squares fit of voxel intensity versus echo time. Including additional shorter echo times could minimize the population of tissue with a zero ms relaxation time and make the T_2^* estimation less sensitive to signal noise. Also, as with any MR study, the tradeoffs between resolution, scan time and image acquisition parameters, such as echo time, must be considered.[26] Due to the relatively small size of the healing ACL, the imaging protocol was optimized to reconstruct the whole volume of the ligament, opposed to a single mid-substance slice,[3,23] and to minimize the influence of partial volume effects. To minimize these concerns we employed high resolution 3-D MR images with minimal voxel size (0.85x0.27x0.27 mm) and no slice gap (0 mm). Also, this study did not include histological confirmation of tissue organization level within the healing ligament. However, a previous meniscal study reported a link between T_2^* and collagen organization.[10] Lastly, the MR images collected in this study were collected postmortem eliminating problems with motion artifact. Future work will incorporate histological confirmation of tissue organization and evaluate the efficacy of using a T_2^* imaging method to predict structural properties *in vivo* for longitudinal studies.

The results reported here provide a critical step toward the improvement of a non-invasive method for predicting the structural properties of a healing ligament for *in vivo* use. Furthermore, the use of relaxation time instead of signal intensity specifically makes this approach independent of image acquisition parameters and is the first step to making this approach comparable across institutions. This will be advantageous for reducing the number of animals used in pre-clinical studies, and provide a noninvasive outcome measure of ligament healing in multicenter clinical trials.

3.5 Conflicts of interest

Two of the authors (MMM, BCF) have patents related to the bio-enhanced ACL repair procedure.

3.6 Acknowledgements

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Chapter 4

T₂* Relaxometry and Volume Predict Semi-Quantitative Histological Scoring of an ACL Bridge-enhanced Primary Repair in a Porcine Model

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Abstract

Magnetic resonance imaging (MRI) variables, such as T_2^* and volume, can predict the healing ligament structural properties. How these MR variables relate to semi-quantitative histology of the healing ACL is yet unknown. We hypothesized that T_2^* and volume would predict the histological scoring of a healing ACL. Yucatan minipigs underwent ACL transection and received: bridge-enhanced ACL repair or no treatment. The surgical legs were harvested after 52 weeks and imaged using a high resolution 2-echo sequence. For each ligament the volume and median T_2^* values were determined. The ACL specimens were then histologically analyzed using the advanced Ligament Maturity Index (LMI). The T_2^* of the healing ligaments significantly predicted the Total LMI score as well as the Cell, Collagen and Vessel sub-scores; $R^2=0.78, 0.67, 0.65$, and 0.60 , respectively ($p<0.001$). The ligament volume also predicted the Total LMI score, Cell and Collagen sub-scores; $R^2=0.39, 0.33, 0.37$, and 0.60 , respectively ($p<0.001$). A lower ligament T_2^* or a higher volume was associated with higher histological scores of the healing ligaments. This study provides a critical step in the development of a non-invasive method to evaluate ligament healing on a microscopic scale.

4.1 Introduction

Current methods to evaluate ligament healing in animal models, such as histological evaluation or biomechanical testing, are crucial for tracking the healing response. However, these methods require biopsy or destructive testing and are not conducive for *in vivo* assessment. Using non-invasive MR imaging to evaluate graft maturation biomechanically, significant correlations were found between signal intensity and structural properties of the ACL reconstruction graft in an animal model.[1,3,16] The combination of both ligament/graft volume (amount of tissue) and signal intensity (tissue quality) significantly improved those predictions.[3] However, signal intensity is not ideal to document ligament healing as it is dependent on MR specifications, including manufacturer, acquisition parameters, and hardware effects that can vary between scanners.[6]

T_2^* relaxation time, another MR variable, correlates with the level of tissue organization,[5,12] is well suited for imaging highly organized collagenous structures,[5,11,17] and is less sensitive to imaging parameters than signal intensity data.[6] Healing ligament volume, as defined by the distributions of T_2^* relaxation times within that ligament, can provide a predictive model of structural properties, where longer T_2^* times are associated with lower structural properties.[2] Longer T_2^* values in the meniscus were also found to be associated with worse biomechanical performance [11] and degeneration,[17] as defined by collagen content accessed via histological grading. While these findings between T_2^* relaxation time and histological outcomes in the meniscus are promising, the relationship of healing ACL T_2^* relaxation time with microscopic evidence remained unknown. More specifically, in both the research and clinical settings, it would be advantageous to understand what histological factors contribute to shorter T_2^* times in the healing ACL.

The Ligament Maturity Index (LMI) has been used to assess histological sections of the early and late stages of healing.[14,15] Recently the LMI scoring system was adapted for later stages in “ligamentization”, where proliferation and remodeling of the tissue are more apparent. The adapted LMI assesses a number of complex factors critical to late stage ligament healing. These factors are evaluated with metrics, which form the Cell, Collagen and Vessel sub-scores and are then combined into the Total LMI score (Table 4.1).[15]

To date there have been no studies evaluating how T_2^* relaxation time and volume of a healing ligament can predict histological scoring. The objective of this study was to test a non-invasive method for predicting the histological outcomes of a bridge-enhanced ACL repair or a naturally healing ligament in a

porcine model at 52 weeks of healing using T_2^* and volume. We hypothesized that MR derived measures of T_2^* and volume would be significant predictors of the histological scoring of a healing ACL after 52 weeks of healing.

4.2 Methods

4.2.1 Animal Model

This controlled laboratory experiment was approved by the Institutional Animal Care and Use Committee. Fifteen Yucatan minipigs (approximately 15 months of age) underwent unilateral ACL transection surgery as previously described.[13] Immediately following transection, eight of the animals received bridge-enhanced ACL repair with an extracellular matrix-blood composite and seven were left untreated to heal naturally without bridge-enhanced repair (ACL transection).[13] It should be noted that these animals were part of another study evaluating the long-term effects of the bridge-enhanced ACL repair.[2,13,15] At 52 weeks, all surgical knees were harvested and immediately imaged.

4.2.2 MR Imaging

All image data were acquired of the surgical knees using a 3T Siemens Tim Trio scanner (Erlangen, Germany) using a 20cm volume extremity coil. T_2^* relaxation time was estimated using high-resolution 3-D gradient echo (FLASH) data sets with parameters: $TR=25ms$, $TE=7.36, 15.24ms$ (2 echoes), flip angle= 12° , $FOV=140mm$, slice thickness= $0.85mm$ (contiguous slices), reconstruction matrix size= 512×512 , 3 averages, bandwidth= $130Hz/pixel$, and scan time= 19 minutes.[2] The bridge-enhanced repaired and naturally healing transected ligaments were manually segmented from the MR images in both the coronal and sagittal planes using commercially available software (Mimics 13.1, Materialise, Ann Arbor, Michigan). 3-D models of the healing ligaments were then created.[3] The total number of ligament voxels was used to determine the volume of the whole ligament (16.1 voxels equaled one mm^3).

Cell Sub-Score (total = 8 pts)		Collagen Sub-Score (total = 12 pts)		Vessel Sub-Score (total = 6 pts)	
Criteria	pts	Criteria	pts	Criteria	pts
Presence of inflammatory cells		Width of bundles		Density of blood vessels	
Necrosis	0	No bundles	0	None present	-1
Polymorphonuclear cells	1	Width less than 50 µm	2	more than 200% present	0
No inflammatory cells	2	Width greater than 50 µm	4	150%-200% present	1
				Less than 150% present	2
Nuclear aspect ratio (NAR) of fibroblasts		Bundle orientation with long axis of ligament		Vessel orientation with long axis of ligament	
No cells	-1	No bundles	-2	No vessels oriented	-2
Average NAR less than 2 (round)	0	less than 50% oriented	0	Less than 30% oriented	-1
Average NAR 2-4	1	50%-75% oriented	2	less than 50% oriented	0
Average NAR greater than 4 (elongated)	2	75%-100% oriented	4	50%-75% oriented	1
				75%-100% oriented	2
Nucleus of fibroblast aligned with fascicles and long axis of ligament		Collagen Crimp		Vessel maturity	
No cells	-2	No crimp	-2	No vessels seen	0
Less than 30% of cells oriented	-1	less than 25% crimp	0	Capillaries only present	1
30%-50% oriented	0	25%-75% crimp	2	Arterioles present	2
50%-75% oriented	1	Crimp with normal length present	4		
75%-100% oriented	2				
Number of fibroblasts					
None	-1				
Greater than 2x normal	0				
Between 1.5x and 2x normal	1				
Less than 1.5x normal	2				

Table 4.1: Criteria used to determine the advanced Ligament Maturity Index (LMI). The five regions from each ligament were separately scored according to the cell, collagen and vessel criteria. The cell, collagen and vessel sub-scores for each ligament were then determined by averaging the scores for each of the five regions using the sub-scores respective criteria. The resulting cell, collagen and vessel sub-scores for each ligament were then summed to determine the total LMI score representing the cumulative indications of healing.

4.2.2.1 T_2^* Estimation

Using previously described methods,[2] Matlab (R2012b; MathWorks, Natick, MA) code was used to estimate T_2^* maps using the signal intensity (SI) relationship:[2,9]

$$T_2^* = \left(\frac{\ln SI_1 - \ln SI_2}{TE_2 - TE_1} \right)^{-1} \quad (4.1)$$

where SI_1 and SI_2 are the signal intensities corresponding to the echo times TE_1 and TE_2 where $TE_2 > TE_1$ for each voxel. The ligament specific maps were produced by extracting the voxels corresponding to the ligament from the T_2^* maps using the 3-D models created from the segmented images. The median T_2^* value was determined for each ligament volume and then compared to the ligament scoring metrics as described below. The distributions of T_2^* for each ligament were found to be lognormal and positively skewed. Median T_2^* was used to represent the central tendency of each ligament as differences in the median value would be representative of the entire ligament distribution.

4.2.3 Histological Scoring

Following imaging, the specimens were frozen and stored at -20 degrees Celsius until mechanical testing. The knees were then prepared and mechanically tested to determine structural properties. [13,15] Following biomechanical testing, knees were cut sagittally through the center of the ACL. Bone-ligament-bone sections were formalin fixed, decalcified (DELTA-Cal, Delta Products Group, Aurora, IL), and paraffin embedded. 7 μ m thick sections were cut with a microtome, placed onto custom glass slides (Corning 75x50 mm Plain Microscope Slides, Corning Incorporated, Corning, NY), and stored at 4° C until staining with hematoxylin and eosin (H&E) or alpha-smooth muscle actin antibodies (SMA).[15] Cell density, morphology, as well as collagen formation (evaluated under polarized light with a 137nm wavelength filter) were assessed on the H&E slides, while SMA stained sections were used to determine vascularity.

Using the advanced LMI (Table 4.1), the sagittal sections from the central portions of the ligaments were scored in five regions; 1mm into the ligament from either tibial or femoral insertion site, and three regions in between. When choosing the five regions for histological analysis, the examiner selected regions that were free of synovium and not visibly deformed by biomechanical testing.[15] The five regions from each ligament were separately scored according to the cell, collagen and vessel criteria. The cell, collagen and vessel sub-scores for each ligament were then determined by averaging the scores for each of the

five regions using the sub-scores respective criteria. The resulting cell, collagen and vessel sub-scores for each ligament were then summed to determine the total LMI score, representing the cumulative effects of healing (Table 4.1). All investigators were blinded to the treatment group for all histological and MRI evaluations. Additionally, the intraclass correlation coefficient between examiners using the Ligament Maturity Index has been shown to be greater than 0.90.[8]

4.2.4 Data Analysis

The repaired and naturally healing transected ligaments were grouped together and analyzed as a single data set. First, single linear regression models were used to separately test the relationships between: 1) ligament median T_2^* and histological scores and, 2) MR volume and histological scores (Total LMI, Cell sub-score, Collagen sub-score, and Vessel sub-score).

Subsequently, both ligament median T_2^* and ACL volume were included in a multiple linear regression model to predict the histological scores. For all regression equations, the R-square values and p-values for the models were reported as indicators of the strength of the relationships. Individual p-values of the independent variables in the multiple regression equation were used to assess the relative contribution of ligament median T_2^* and volume to the prediction. 95% confidence intervals (Figure 4.1) and standard error (SE) (Tables 4.2 and 4.3) of the regression equations were also determined as a means to assess the accuracy of the prediction equations.

4.3 Results

The median ligament T_2^* of bridge-enhanced repaired and naturally healing transected ligaments significantly predicted Total LMI score as well as Cell, Collagen and Vessel sub-scores; $R^2=0.78, 0.67, 0.65$, and 0.60 , respectively (all $p<0.001$) (Figure 4.1, Table 4.2). The MR ligament volume was also a predictor of Total LMI score as well as Cell, and Collagen sub-scores; $R^2=0.39, 0.33$, and 0.37 , respectively ($p=0.012, p=0.025$, and $p=0.016$) (Figure 4.1, Table 4.2). Ligament volume only approached significance for predicting the Vessel sub-score; $R^2=0.25$, $p\text{-value}=0.059$ (Figure 4.1, Table 4.2). In general a lower median ligament T_2^* or a higher ligament volume was associated with indications of healing or higher histological scores of the repaired and transected ligaments (Figures 4.1, 4.2, 4.3).

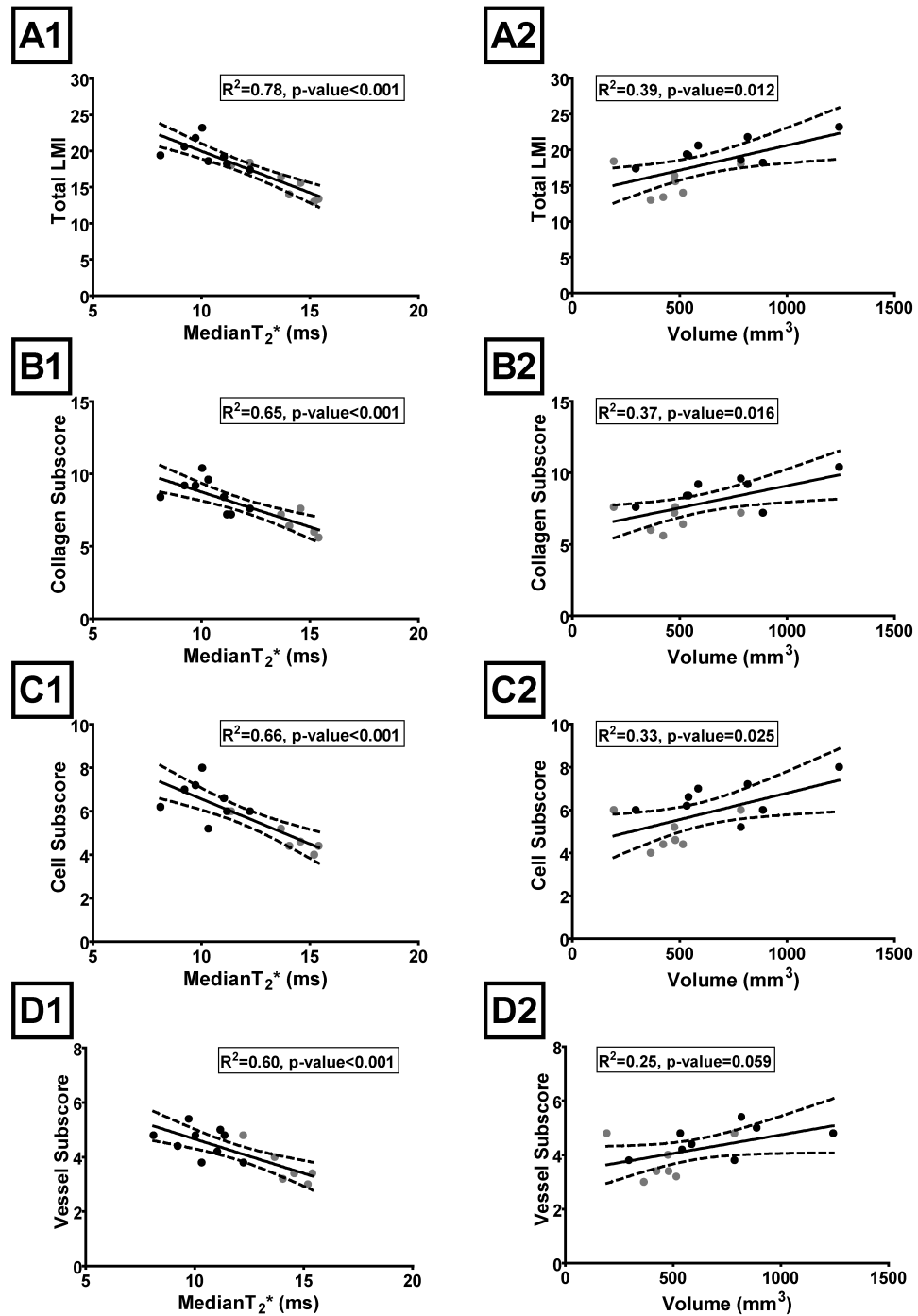


Figure 4.1: The healing ligament histology scores (A- Total LMI, B- Collagen Sub-score, C- Cell Sub-score, and D- Vessel Sub-score) as a function of ligament median T_2^* value (A1, B1, C1, and D1) and volume (A2, B2, C2, and D2) in the linear regression models. Dashed lines represent 95% confidence interval. The ligaments that received bridge-enhanced ACL repair are depicted with black circles and ligaments that were transected and left to heal naturally are depicted with gray circles.

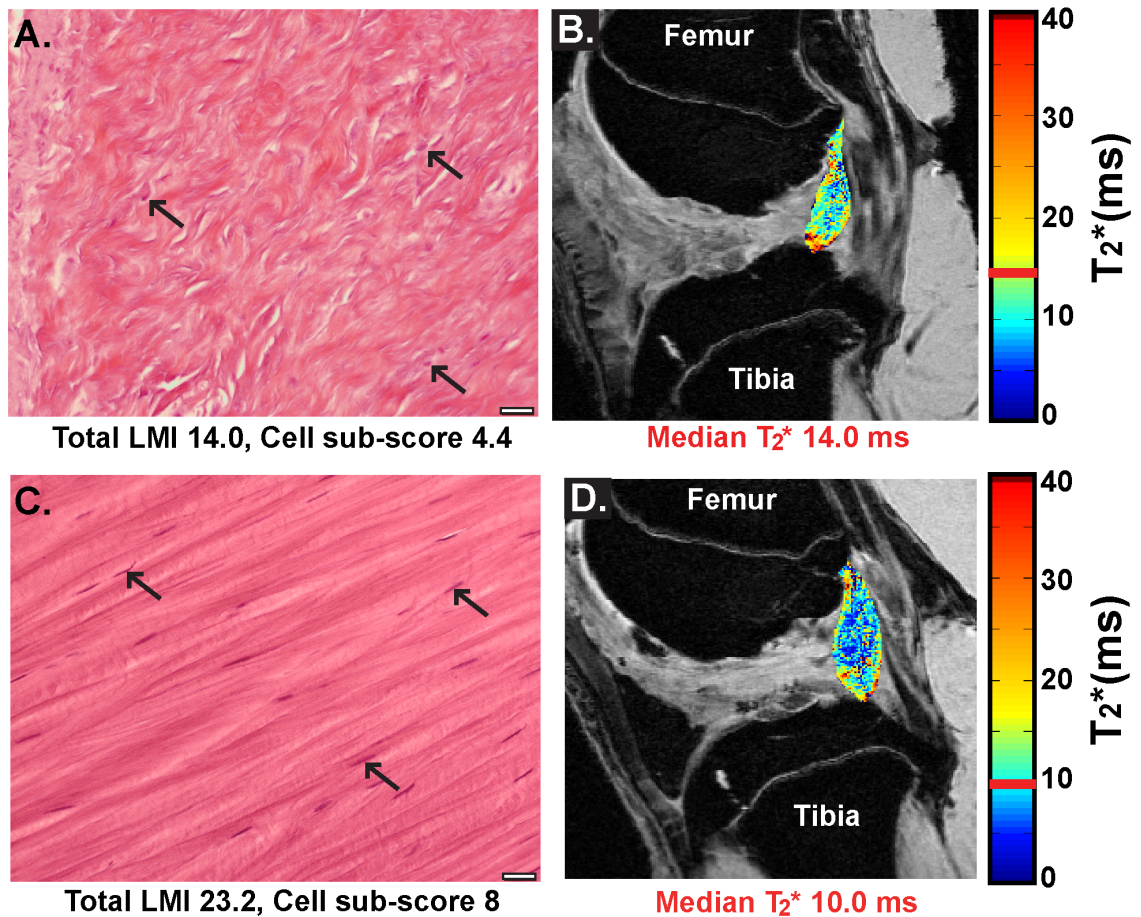


Figure 4.2: A) Example ligament histology image with a low total ligament score and cell sub-score (Total LMI 14.0, Cell sub-score 4.4). Arrows indicate cell nuclei not clearly aligned with longitudinal axis of collagen fibers. Also note the collagen fibers lack a distinct longitudinal axis. B) The associated T_2^* ligament map for the low total LMI histology image overlaid on the original DICOM image. C) Example ligament histology image with a high total ligament score and cell sub-score (Total LMI 23.2, Cell sub-score 8). Arrows indicate cell nuclei aligned with longitudinal axis of collagen fibers. D) The associated T_2^* ligament map for the high total LMI histology image overlaid on the original DICOM image. Histology images are H&E stained at 40X magnification, scale bar indicates 20 microns. The color bars in the T_2^* maps represent the range of T_2^* values in the ligament with the median T_2^* value for the ligament highlighted in red. The MR images are a sagittal view of the femoral notch with the femur at the top of the image and the tibia at the bottom. For the MR images shown $TE = 7.36$ ms.

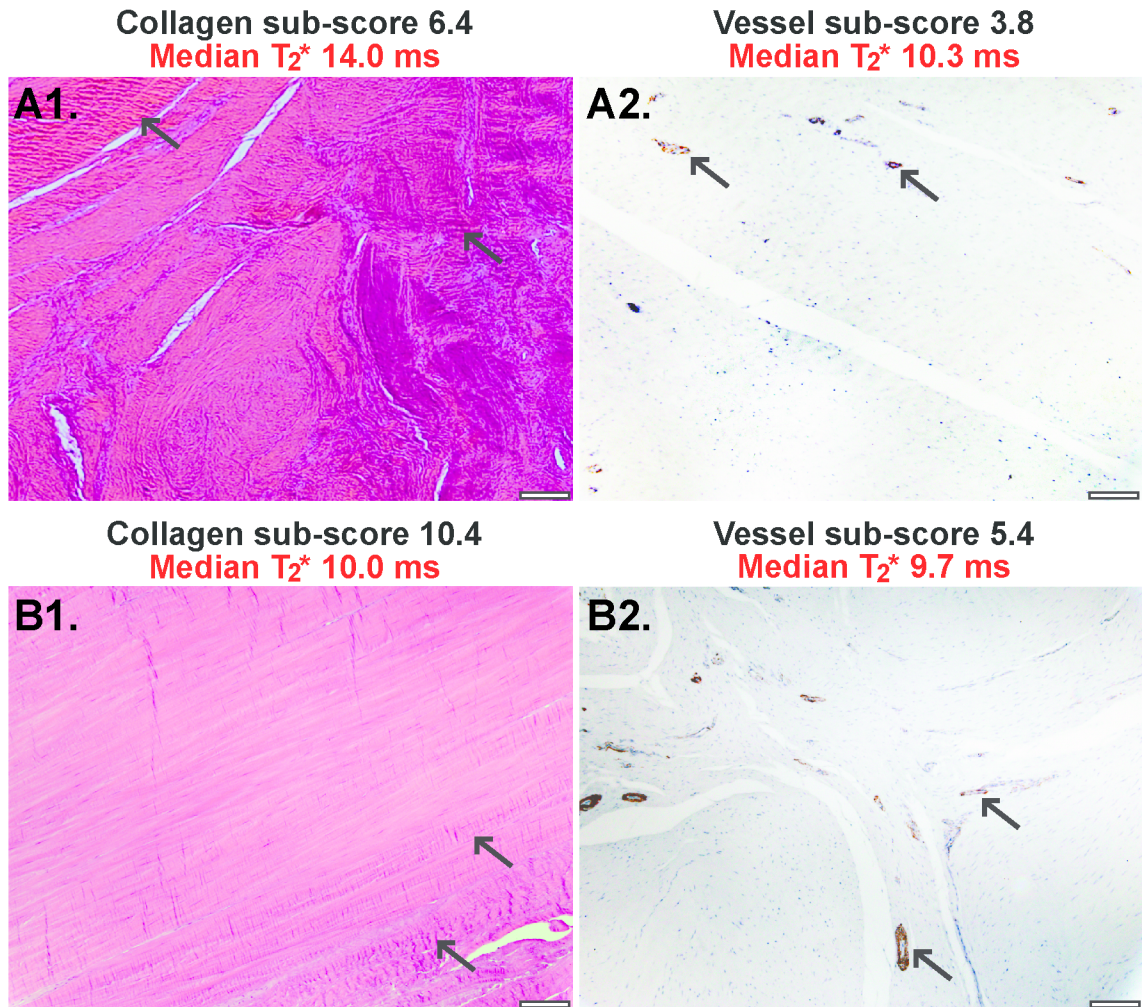


Figure 4.3: A1) Example H&E stained polarized image with a collagen sub-score of 6.4 and median ligament T_2^* of 14 ms. Arrows indicate areas with collagen crimp not distinctly aligned with fiber longitudinal axis. A2) Example SMA stained image with a vessel sub-score of 3.8 and median ligament T_2^* of 10.3 ms. Arrows indicate smooth muscle like actin rich muscularis layer around arterioles visible in the interfascicular regions. B1) Example H&E stained polarized image with a collagen sub-score of 10.4 and median ligament T_2^* of 10 ms. Arrows indicate collagen crimp aligned with fiber longitudinal axis. B2) Example SMA stained image with a vessel sub-score of 5.4 and a median ligament T_2^* of 9.7 ms. Arrows indicate smooth muscle like actin rich muscularis layer around arterioles visible in the interfascicular regions. All histological images at 10X magnification, scale bars indicate 100 microns.

Using ligament median T_2^* in conjunction with volume, the multiple linear regression model predictions for Total LMI score as well as Cell, Collagen and Vessel sub-scores were marginally improved; $R^2=0.82$, 0.70, 0.71, and 0.61, respectively (all $p<0.001$) (Table 4.3).

4.4 Discussion

A quantitative tool for assessing the healing of an ACL post-operatively could be valuable to both researchers and clinicians. Ligament T_2^* and volume has been used to assess structural properties of a healing ligament,[2] but has not yet been used to quantitatively assess ligament healing via histology. In this study, the MR derived terms of median ligament T_2^* and volume significantly predicted the semi-quantitative histological scores in healing bridge-enhanced repaired and natural healing transected ligaments. In general, a shorter median T_2^* time and larger volume were associated with better histological outcome scores (i.e., improved healing) (Figure 4.2, 4.3). As expected, the MR ligament volume was not as strongly associated with histological outcomes as median T_2^* as indicated by respective p-values and R^2 values of the single regressions (Table 4.2). This difference in prediction strength between median T_2^* and volume is likely due to the microscopic nature of the histological assessments. Few of the histological evaluation criteria account for gross ligament size, while the majority of the criteria relate to elements of tissue organization and remodeling and it is these factors that have been found to effect T_2^* times.[5,12,17] Furthermore, we found that by combining the median ligament T_2^* and MR volume in a multiple regression equation (Table 4.3), the ability to predict the histological outcome scores was only marginally improved as observed by the small R^2 increases between the single and the multiple linear regression models. This marginal improvement in the multiple regression model is due to the T_2^* term dominating the regression prediction and the volume data having a weaker association with the histological outcome criteria, which can be observed by the higher individual p-values for volume from the multiple regression analysis (Table 4.3).

Using ligament median T_2^* to predict the Total LMI score ($R^2=0.78$, $p\text{-value}<0.001$) was stronger than the T_2^* prediction of individual sub-scores (Cell $R^2=0.67$, $p\text{-value}<0.001$; Collagen $R^2=0.65$, $p\text{-value}<0.001$; and Vessel $R^2=0.60$, $p\text{-value}<0.001$)(Table 4.2). This difference in prediction strength is a result of the cumulative nature of the Total LMI score, which accounts for the complexity of the cellular, collagen and vascular aspects of the healing process for the whole ligament. Furthermore, the Total LMI assesses healing throughout five different regions of the ligament, and as such, we would expect it to relate to the median ligament T_2^* , a measure representing the whole ligament.

Histology Scoring Dependant Variable	Model	MR Independent Variable	Coefficient	p-value	SE of the estimate	R ²
Total LMI	Single Regression	Median T ₂ *	-1.15	<0.001	1.4	0.78
		Volume	0.0069	0.012	2.3	0.39
Cell Sub-score	Single Regression	Median T ₂ *	-0.415	<0.001	0.7	0.67
		Volume	0.002	0.025	1	0.33
Collagen Sub-score	Single Regression	Median T ₂ *	-0.49	<0.001	0.6	0.65
		Volume	0.003	0.016	1.1	0.37
Vessel Sub-score	Single Regression	Median T ₂ *	-0.25	<0.001	0.6	0.60
		Volume	0.001	0.059	0.7	0.25

Table 4.2: Summary of the healing ligament histology score single linear regression prediction equations as a function of ligament median T₂^{*} value or volume.

Our findings align with a prior MR investigation looking at signal to noise quotient (a measure of SI) in healing ACL grafts using an ovine model.[16] Qualitative histological assessment of graft healing was found to be present with grafts displaying a lower SI and higher structural properties.[16] The qualitative nature of the histological assessments in this previous study did not allow for a direct prediction analysis. Building on these results, we found that by using T₂^{*} relaxation time we could predict a semi-quantitative histological score (LMI) for assessing ligament healing and maturation. Additionally, the relationship between our MR variable T₂^{*} and histological scores tells a similar narrative to the qualitative findings of the previous ovine study. We found low T₂^{*} values were associated with greater cell density and collagen organization where the previous study found similar histological results for grafts with low SI.

MR imaging has also been used to find a correlation between T₂^{*} and level of collagen organization assessed with semi-quantitative histology in the meniscus. Short T₂^{*} times were identified with more densely packed collagen fibers and longer T₂^{*} values were associated with less densely packed collagen and meniscal damage.[17] While our study used a different means to acquire T₂^{*} times, linking collagen structure with shorter T₂^{*} values aligns with the findings presented herein. This further suggests that T₂^{*} can serve as a proxy for tissue organization and that remodeling in healing ligaments can be identified with these MR methods.

Using only two echo times adds uncertainty to the determination of T₂^{*} by overestimating the relaxation time value.[6,10] However, a two echo estimation has been shown to correlate with a six echo determina-

Histology Scoring Dependant Variable	Model	MR Independent Variable	Coefficient	Individual p-value	Regression p-value	SE of the estimate	Regression R ²
<i>Total LMI</i>	<i>Multi Regress</i>	<i>Median T₂*</i>	-0.99	<0.001	<0.001	0.8	0.82
		<i>Volume</i>	0.0027	0.106			
<i>Cell Sub-score</i>	<i>Multi Regress</i>	<i>Median T₂*</i>	-0.36	0.002	<0.001	0.7	0.70
		<i>Volume</i>	0.0009	0.254			
<i>Collagen Sub-score</i>	<i>Multi Regress</i>	<i>Median T₂*</i>	-0.40	0.003	<0.001	0.8	0.71
		<i>Volume</i>	0.0014	0.159			
<i>Vessel Sub-score</i>	<i>Multi Regress</i>	<i>Median T₂*</i>	-0.23	0.006	<0.001	0.5	0.61
		<i>Volume</i>	0.0004	0.500			

Table 4.3: Summary of the healing ligament histology score single linear regression prediction equations as a function of ligament median T₂* value and volume.

tion of T₂*, indicating the T₂* estimation used here captures the inter-specimen relative differences.[4] Additionally, this approach served to limit scan time (19 min) and post-processing time, important factors to consider for eventual clinical translation.[4,7,9] Histological scoring was performed after a freeze-thaw cycle, to minimize potential differential effects of freezing on the histological findings of the ligaments all knees were subject to the same freeze thaw protocol. Preceding histological analysis, mechanical testing was performed.[15] However, a quasi-static protocol was used to test the ligaments (ramp speed 20mm/min) allowing the testing to be halted once a drop in load occurred to avoid complete disruption of the ligament and to minimize irreversible changes during the mechanical testing. All ligaments in this study failed within the midsubstance allowing histological assessment of the areas out side the area of local disruption. It is also reasonable to assume that the failure testing did not alter cellular and vascular content. For this study, we assumed that median graft T₂* and volume were the only MR variables that would account for LMI histologic scores. It is possible that other MR analyses, such as diffusion weighted imaging to quantify fiber alignment, could improve correlations to histology. Lastly, the MR images collected in this study were collected postmortem eliminating problems with motion artifact. Future work will evaluate the efficacy of using a T₂* imaging method to predict structural properties *in vivo* for longitudinal studies.

In this study, median ligament T₂* and MR volume were found to be predictive of semi-quantitative histological scores assessing healing of the ACL in a porcine model at 52 weeks post operatively. This

study provides a critical step in the development of a non-invasive method to predict healing on a microscopic level for bridge-enhanced repair. This technique may prove beneficial as a surrogate outcome measure for healing and may have clinical implications for tracking post-operative changes with ligament healing in patient cohort studies and randomized controlled trials.

4.5 Conflicts of interest

Two of the authors (MMM, BCF) have patents related to bridge-enhanced ACL repair.

4.6 Acknowledgements

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Chapter 5

Improving the Clinical Efficiency of T_2^* Mapping of Ligament Integrity

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Abstract

Current MR methods use T_2^* relaxation time as a surrogate measure of ligament strength. Currently, a multi-echo voxel-wise least squares fit is the gold standard to create T_2^* maps; however, the post-processing is time-intensive and serves as a stopgap for clinical use. The study objective was to determine if an alternative method could improve post-processing time without sacrificing fidelity of T_2^* values for eventual translational use in the clinic. Using a 6 echo FLASH sequence, three different methods were used to determine intact posterior cruciate ligament (PCL) median T_2^* . Two of these methods utilized a voxel-wise method to establish T_2^* maps: 1) a current "gold standard" method using a voxel-wise 6 echo least-squares fit (6LS) and 2) a voxel-wise 2 echo point T_2^* determination (2MM). The third method used median ligament signal intensity and a single nonlinear least-squares fit (6LS_{ROI}) instead of a voxel-wise basis. The resulting median T_2^* values of the PCL and computational time were compared. The median T_2^* values were 42% higher using the 2MM compared to the 6LS method ($p < 0.0001$). However, a strong correlation was found for the median T_2^* values between the 2MM and 6LS methods ($R^2 = 0.80$). The median T_2^* values were not significantly different between the 6LS and 6LS_{ROI} methods ($p = 0.519$). Using the 2MM (which provides a regional map) and the 6LS_{ROI} (which efficiently provides the median T_2^* value) methods in tandem would take only minutes of post-processing computational time compared to the 6LS method (~540 minutes), and hence would facilitate clinical application of T_2^* maps to predict ligament structural properties as a patient outcome measure.

Keywords: T_2^* relaxometry, MRI, Posterior Cruciate Ligament

5.1 Introduction

Non-invasive evaluation of a ligament's structural properties using magnetic resonance imaging (MRI) would be valuable for quantifying tissue healing in research and clinical settings. Current methods use either signal intensity (SI) [1] or T_2^* relaxation time [2] as surrogate measures of ligament strength. While SI measures have been successfully used to predict ligament strength in terms of structural properties (maximum load, yield load, linear stiffness) [1,12], these SI measures are dependent on MR scan parameters and can vary between scan sessions and manufacturers, making cross-institutional studies or development of a universal assessment standard difficult [3].

Alternatively, T_2^* is an inherent tissue property, is less sensitive to scan parameters, and has been shown to be reproducible across similar strength magnets and sites [3]. However, current T_2^* methods involve acquiring multiple-echo sequences at high-resolution. These three-dimensional, high-resolution images are essential for accurately characterizing small structures like the posterior cruciate ligament (PCL). In a research setting, T_2^* maps generated from these high-resolution images are critical for identifying potential pathology and mapping regional variations as they relate to the biomechanical properties within a ligament [2]. Currently, a voxel-wise multi-echo least squares fit is the gold standard to create T_2^* maps [6]. Multi-echo least squares fit relaxometry maps have been proven to be accurate, minimally sensitive to noise [7] and helpful to visualize regions of interest (ROI). Unfortunately, the post-processing associated with a least-squares fit is time-intensive and thus difficult to implement clinically. While post-processing time can be reduced by decreasing total voxel number, high resolution scans are required to properly characterize small ligament structures. However, the T_2^* fitting function could be modified to make it time appropriate for a clinical setting without decreasing resolution. One method would be to use only two echo times [6], allowing for the point determination of T_2^* using matrix math (2MM), which would be many magnitudes faster than the least squares fit algorithm [2,4]. However, two-point estimations have been shown to overestimate relaxation times [3,8].

A completely different method is theoretically possible. For most relaxometry studies, a ROI is extracted from the full field of view (FOV) T_2^* map and simple summary statistics (such as median and quartiles) are used to summarize the ligament T_2^* values. These summary statistics are important as quantifiable surrogate measures of structural properties [2,9] and to characterize tissue homogeneity. However, the summary is done after the time-intensive post-processing required to generate the T_2^* maps. A different method would be to extract the median ligament SI for the ROI at each echo time and then use a nonlinear least-squares fit to directly calculate median T_2^* from the ROI median SI values (6LS_{ROI})

[5]. Despite being many magnitudes faster than the voxel-wise gold standard method, this T_2^* post processing approach needs to be validated for ligament tissue on a 3T magnet.

The purpose of this study was to validate two alternative methods of T_2^* determination (2MM and 6LS_{ROI}) in comparison to a current gold standard (6LS) by determining the differences in fidelity and post-processing time between the methods. We hypothesized that there would be a strong correlation between median T_2^* values for 2-echo point estimation (2MM) and voxel-wise six-echo nonlinear least squares fit gold standard (6LS). We also hypothesized that there would be no significant difference between the gold standard (6LS) and ROI (6LS_{ROI}) median T_2^* values for each ligament, and that there would be a strong correlation between gold standard (6LS) and 6LS_{ROI} T_2^* quartile values. These two alternative approaches (2MM and 6LS_{ROI}), used in tandem, would take significantly less post-processing time than the gold standard, and could be combined to offer a tool for assessing ligament structural properties.

5.2 Methods

With IACUC approval, 12 adult sheep underwent unilateral ACL transection surgery followed by bio-enhanced ACL repair as previously described [10]. After 20 weeks of healing, the animals were euthanized and the knees were harvested.

5.2.1 Imaging

Using the FLASH sequence, a high resolution three-dimensional data set, utilizing 6 echo times, (TR/TE/FA, 33/4.3, 7.3, 10.2, 13.1, 16.0 & 18.9/ 17°; FOV, 180 mm; matrix 512X512, slice thickness/gap, 0.8mm/0; avg 1; bandwidth 407) was acquired of the injured knee on a 3T scanner (Siemens Trio), immediately after limb harvest. The total scan time was 19 minutes. The intact PCL in the operative joint was then segmented from the image stack (Mimics 15.0; Materialize, Ann Arbor, MI) and three-dimensional models were created [1,2] to establish ligament ROI. To create a worst-case scenario for detecting differences in the computation of T_2^* across the three different computational methods, the intact PCL was chosen as a standard of comparison to reduce variability in the MR variables T_2^* and volume. The same MR data set, ROIs, programming language (MatLab: MathWorks, Natick, MA) and computational hardware (Intel Xeon E5540 Processor, 2 Cores, 2.53 GHz, 16 GB RAM, OSCAR High-Performance Computing

Cluster, Brown University) were used for all 3 post processing methods to determine T_2^* of the intact PCL.

5.2.2 Post processing T_2^* determination

5.2.2.1 Gold standard: 6 echo least squares fit T_2^* Map (6LS)

For the gold standard T_2^* map, a voxel-wise nonlinear least-squares fit of voxel SI versus echo time for T_2^* estimation (6LS) was used. SI from all six echo times along with the SI relationship,

$$SI(TE) = M_0 e^{-TE/T_2^*} + DC \quad (5.1)$$

where $SI(TE)$ are the voxel specific SIs for the various echo times (TE). The three fit parameters are M_0 (equilibrium magnetization), T_2^* and the DC offset (DC), which were used for the least squares fit estimation of T_2^* [6].

5.2.2.2 Two Echo determination T_2^* Map (2MM)

For the first alternative method, SI from the 7.3 and 16.0 ms echo times [2] (TE) along with the SI relationship, [6]

$$T_2^* = \left(\frac{\ln SI_1 - \ln SI_2}{TE_2 - TE_1} \right)^{-1} \quad (5.2)$$

where SI_1 and SI_2 are the SIs corresponding to the echo times TE_1 and TE_2 and where $TE_2 > TE_1$ for each voxel, was used for a 2-point estimation of T_2^* . The PCL voxels were extracted from both the 2MM and 6LS maps using the ligament ROIs (Figure 5.1). From these ligament specific maps, histograms of the voxel-wise T_2^* were plotted for both the 6LS and 2MM method. From each ligament's histogram, summary statistics (median, 1st quartile and 3rd quartile) were calculated for the 6LS and 2MM methods.

5.2.2.3 SI Region of Interest Median T_2^* (6LS_{ROI})

For the second alternative method, the SI voxels corresponding to the ligament were extracted from all 6 echo times using the ligament ROIs. The SI summary statistics were then calculated for each ROI at each echo time. The median SI summary statistic along with the relationship,

$$SI_{Median}(TE) = M_0 e^{-TE/T_2^*_{Median}} + DC \quad (5.3)$$

where SI_{median} represents the median ROI SI for the various echo times (TE), and the three fit parameters are the ligament's median M_0 , T_2^* and DC offset (DC), were used for a least squares fit estimation of median T_2^* . The same was done for the first and third quartile T_2^* summary statistics.

5.2.3 Statistics

The statistical differences of the summary statistics between the 2MM and 6LS methods, as well as between the 6LS and 6LS_{ROI} were tested using paired t-tests with Bonferroni correction. The linear relationships between the 2MM and 6LS methods and between the 6LS_{ROI} median T_2^* and the 6LS were tested using linear regression. Finally, as a general measure of clinical feasibility, the processing time was compared between methods.

5.3 Results

The T_2^* values (median, 1st quartile and 3rd quartile) were significantly higher with the 2MM method than the 6LS method ($p < 0.0001$) (Figure 5.1, Table 5.1). The median T_2^* value of the 2MM method was, on average, 41.9% greater than that of the 6LS method. There was a strong linear relationship between the 2MM and 6LS median T_2^* values ($p < 0.0001$) ($R^2 = 0.80$) (Figure 5.2, Table 5.1). A full FOV T_2^* map was not available with the 6LS_{ROI} method because the T_2^* values were not calculated on a voxel-wise basis. The median T_2^* values were not significantly different between the 6LS than the 6LS_{ROI} methods ($p = 0.519$) (Table 5.1, Figure 5.1). The 1st and 3rd quartile T_2^* values were significantly different between the 6LS and the 6LS_{ROI} method ($p \leq 0.001$), and had a strong linear relationship ($p < 0.0001$ for both) ($R^2 = 0.88$ and 0.66 respectively) (Table 5.1). The average processing

time for the 6LS, 2MM and 6LS_{ROI} methods was ~540, < 1, < 1 minutes, respectively (Figure 5.1, Table 5.1).

5.4 Discussion

As expected [3], the median and quartile T_2^* values for the 2MM method were significantly higher than the 6LS method, due to the natural log and exponential forms of the 2MM and 6LS methods, respectively. This indicates the absolute T_2^* values derived from the 2MM method will be overestimated compared to values derived using a least squares fit function. However, the highly significant correlation between the 2MM and the 6LS (gold standard) methods (Figure 5.2) indicates the the two methods offer similar relative insight into tissue integrity. This signifies that the relative distribution of T_2^* values in the 6LS generated map can be visualized with the 2MM map [2]. This could make the 2MM T_2^* maps helpful for identifying potential pathology and regional variations as they relate to the biomechanical properties within a ligament (Figure 5.1).

As hypothesized, there was no statistical difference between T_2^* median values for the 6LS and 6LS_{ROI} methods, signifying the 6LS_{ROI} method could be used to calculate a median T_2^* value for a ligament. While the quartile values were statistically different between the 6LS and 6LS_{ROI} methods, the strong linear relationship between them ($R^2=0.88$ and 0.66 for Q1 and Q2, respectively) indicates that the findings seen with the gold standard 6LS method would be observed with the 6LS_{ROI} method. This strong linear relationship is critical because ROI statistics provide another tool for characterizing structural properties [1], homogeneity and potential pathology within a tissue.

At approximately 540 minutes, the magnitude of computational time for the gold standard method would be a stop gap for transitioning this method to both clinical and research settings. Separately, the 2MM and 6LS_{ROI} methods, despite being computationally efficient (< 1 minute), have limitations. The 2MM, while having an inter-specimen relationship similar to the gold standard, overestimates T_2^* , while the 6LS_{ROI} method does not provide a T_2^* map for viewing pathology within a ligament. However, as previously mentioned, the strong linear relationship between the median T_2^* values for the 2MM and 6LS methods indicates that the predictable difference between their T_2^* values could make the 2MM method effective for viewing relative regional variations within structures (Figure 5.1). Therefore, a combination

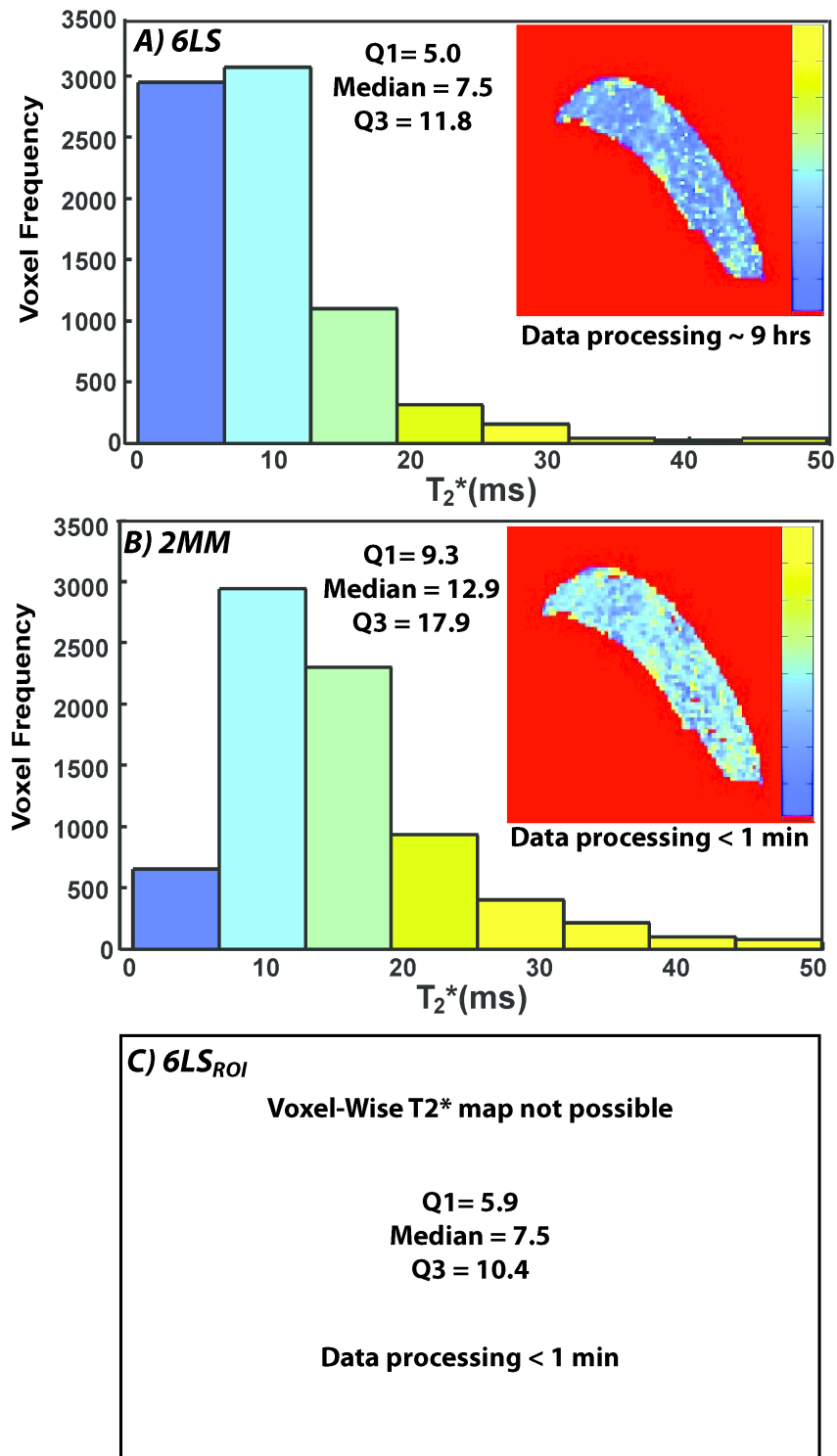


Figure 5.1: Example T_2^* histograms and sagittal ligament ROI maps of the intact PCL, A) determined using a voxel-wise 6 echo least squares fit (6LS) and B) determined using a voxel-wise 2 echo point determination (2MM). C) Median, Q1, Q3 T_2^* determined using the 6LS_{ROI} method, no histogram or map available with this method.

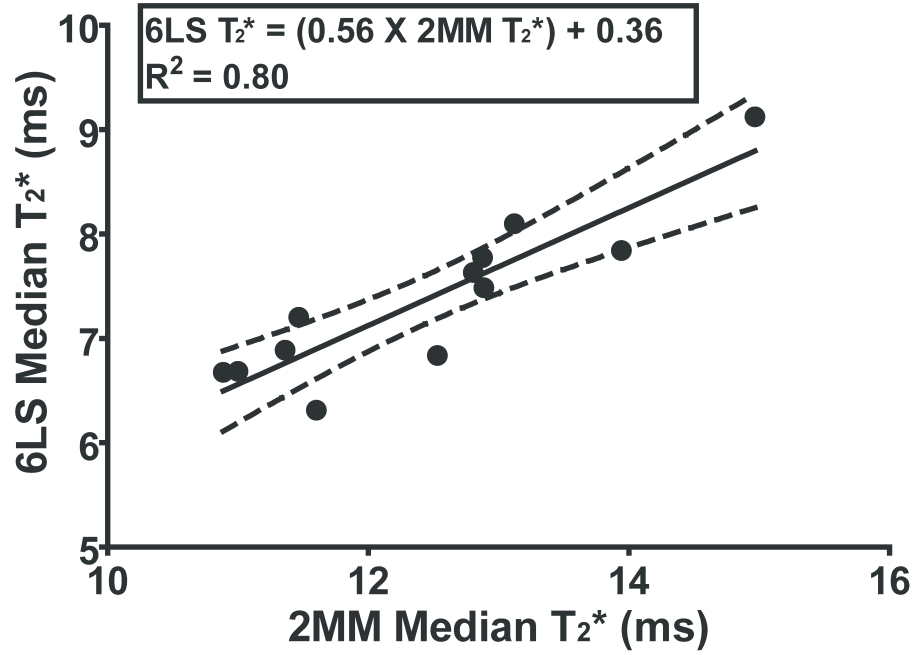


Figure 5.2: Linear regression of 2MM median T_2^* values vs 6LS median T_2^* values

of the 2MM method (which provides the regional map for viewing pathology and quality control) and the 6LS_{ROI} protocol (which provides the median T_2^* value for quantifying tissue integrity) could provide clinics with a powerful ligament structural properties assesment tool requiring minimal computational time.

Summary Stat	Relationship	Paired t-test	R^2
Median	2MM/6LS	($P \leq 0.001$) *	0.8 ($P \leq 0.001$)
Median	6LS _{ROI} /6LS	($P = 0.519$)	0.84 ($P \leq 0.001$)
Q1	6LS _{ROI} /6LS	($P \leq 0.001$) *	0.88 ($P \leq 0.001$)
Q3	6LS _{ROI} /6LS	($P \leq 0.001$) *	0.66 ($P \leq 0.001$)

*Statistically significant differences

Table 5.1: Summary Statistics for different T_2^* determination methods comparison.

This study is limited in that the images were acquired post mortem. Future work will evaluate how this relationship changes *in vivo*. Additionally, the post processing times would vary due to computer hardware, processing software and code optimization. However, even with increases in computational speed or efficiency, the time for 6LS method will remain orders of magnitude longer, reducing its clinical feasibility. Furthermore, 6 echos were chosen to perform the multi-echo least-squares fit, though previous relaxometry studies can vary from 2 to 12 or more echos [4,11]. 6 echos was chosen as the gold standard to balance accuracy, scan time (19 minutes/specimen) and post-processing time.

Assessing T_2^* values of a ROI to determine ligament structural properties is very useful in a research setting [9,13]. However, time intensive post processing could stop translation to clinical use. While the application of the 2MM and the 6LS_{ROI} methods are by themselves limited, using a combination of the 2MM map and the 6LS_{ROI} median T_2^* values (combined post processing time < 2 minutes) instead of the traditional voxel-wise 6LS method (post processing time ~540 minutes) could be a viable option to improve processing time and could be valuable clinically.

5.5 Conflicts of interest

None of the authors have any conflict of interest to report

5.6 Acknowledgements

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Chapter 6

The Uncertainty of Predicting Intact Anterior Cruciate Ligament Degeneration in Terms of Structural Properties Using T_2^* Relaxometry in a Human Cadaveric Model

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Abstract

The combination of healing anterior cruciate ligament (ACL) volume and the distributions of T_2^* relaxation times within it have been shown to predict the biomechanical failure properties in a porcine model. This MR-based prediction model has not yet been used to assess ligament degeneration in the aging human knee. Using a set of 15 human cadaveric knees of varying ages, we obtained *in situ* MR measures of volume and T_2^* of the intact ACL and then related these MR variables to biomechanical outcomes (maximum and yield loads, linear stiffness) obtained via *ex vivo* failure testing. Using volume in conjunction with the median T_2^* value, the multiple linear regression model did not predict maximum failure load for the intact human ACL; $R^2=0.23$, $p=0.200$. Similar insignificant results were found for yield load and linear stiffness. Naturally restricted distributions of the intact ligament volume and T_2^* (demonstrated by the respective Z-scores) in an older cadaveric population were the likely reason for the insignificant results. These restricted distributions may negatively affect the ability to detect a correlation when one exists. Further research is necessary to understand the relationship of MRI variables and ligament degeneration. While this study failed to find a significant prediction of human biomechanical outcome using these MR variables, with further research, an MR-based approach may offer a tool to longitudinally assess changes in cruciate ligament degradation.

Keywords: MRI, ligament degeneration, ACL, structural properties, biomechanics

6.1 Introduction

Osteoarthritis (OA) is a degenerative condition that affects articular cartilage as well as other structures of the joint, including the anterior cruciate (ACL) and posterior cruciate ligaments (PCL) [8,18]. Total knee arthroplasty (TKA) is the most common surgical intervention to treat advanced stages of OA, where it is common to remove the ACL and PCL [5,6]. The newest TKA implant designs allow retention of both ACL and PCL; thus, it would be beneficial to non-invasively assess the structural integrity of the cruciates by predicting their biomechanical properties in order to inform surgical decision making in the selection of prostheses for patients undergoing TKA.

Using MR images, it has been shown that the combination of ligament volume (amount of ligament tissue) and signal intensity (surrogate of tissue quality) is predictive of the biomechanical failure properties of a healing ligament in a porcine model [3,16]. More recently, the combination of healing ligament volume and the distributions of T_2^* relaxation times within the ligament have been found to provide a more robust prediction model of the structural properties [2].

To date this MR-based prediction model has not yet been used to assess ligament degeneration in the aging knee. Using human cadaveric knees, the objectives of this study were to obtain *in situ* MR measures of volume and T_2^* of the intact ACL and to relate these variables to biomechanical failure outcomes (maximum and yield loads, linear stiffness) obtained *ex vivo*. We hypothesized that the MR values of volume and T_2^* of the intact ACL would predict the ligament structural properties. Such an MR-based approach would offer a valuable tool to longitudinally assess changes in cruciate ligament degeneration, and to assess cruciate ligament integrity when selecting TKA prosthesis type.

6.2 Methods

6.2.1 Cadaveric Specimens

Fifteen frozen human cadaver knees (5 female and 10 male, Table 6.1) were thawed to room temperature and imaged *in situ*. The age distribution (range 24-76) was selected to capture a range of ligament degeneration states, as the structural properties of the ligament have been shown to decrease with age [18]. After MR imaging the specimens were frozen until mechanical testing.

6.2.2 MR Imaging

All image data were acquired using a 3T Siemens Tim Trio scanner (Erlangen, Germany) using a 20cm volume extremity coil. High-resolution 3-D gradient echo (FLASH) data sets were obtained with parameters: TR=33ms, TE=4.3, 7.3, 10.2, 13.1, 16ms (6 echoes), flip angle=17°, FOV=180mm, slice thickness=0.8mm, reconstruction matrix size=512x512, slice thickness=0.8mm (contiguous slices), single average, and bandwidth=407Hz/pixel [2]. The intact ACLs were manually segmented from the image stack by one examiner with 5 years of experience and 3-D models of the ligaments were created (Mimics 15.0; Materialize, Ann Arbor, MI) [2,3]. MatLab (MathWorks, Natick, MA) was used to determine T_2^* maps of the ACL for each knee.

6.2.2.1 Post Processing: Intact Ligament T_2^* determination

To create the T_2^* map for each knee, a voxel-wise nonlinear least-squares fit of voxel signal intensity (SI) versus echo time was used. SI from all six echo times along with the SI relationship,

$$SI(TE) = M_0 e^{-TE/T_2^*} + DC \quad (6.1)$$

where SI(TE) are the voxel specific SIs for the various echo times (TE), was used to estimate T_2^* . The three fit parameters M_0 (equilibrium magnetization), T_2^* and the DC offset (DC), were used for the least squares fit estimation of T_2^* [4,10]. To isolate ligament specific T_2^* values, the voxels corresponding to the ligament were extracted from the T_2^* maps using the 3-D models created from the segmented images [2,3]. Summing the total number of ACL voxels provided an estimate of the whole ligament volume (VOL) (10.2 voxels equaled one mm³).

6.2.3 Structural Properties

An established tensile testing protocol was used to determine the structural properties of the intact ACLs [14]. The specimens were thawed to room temperature. The proximal end of the femur and the distal end of the tibia were potted in PVC pipe using a urethane resin (Smooth-On, Easton, Pennsylvania). The

joint was carefully dissected, leaving only the femur-ACL-tibia complex intact. Using a servohydraulic material testing system (MTS 810; Prairie Eden, MN), tensile loads were applied at 20 mm/min to failure [14]. The entire tensile load-displacement curve was recorded until a precipitous drop in load occurred. The maximum load, yield load, and linear stiffness values of the ligaments were calculated from the load-displacement data.

6.2.4 Statistics

Linear regression models were used to separately test the relationships between ligament: a) MR volume and structural properties and, b) T_2^* and structural properties. Subsequently, both ligament volume and T_2^* were included in a multiple linear regression model to predict the structural properties (SigmaPlot 12.0; Systat Software Inc., San Jose, CA)(Figure 6.1). The R-square, standard error and p-values were reported as indicators of the relationship strength and goodness of fit. Descriptive statistics such as maximum, minimum, median, 25, 75% confidence intervals and kurtosis (a variable indicating the level of data clustering) [1] were used to assess the shape and distribution of the independent variables (volume, median T_2^*) (Figure 6.2, Table 6.3). To visualize the shape and distribution of the independent variables, volume and median ligament T_2^* were transformed to Z-scores and plotted as histograms (Figure 6.2). Z-scores indicated the number of standard deviations from the mean of a data set a data point is [1].

6.3 Results

6.3.1 Regression Prediction Model

The volume of intact human cadaveric ACLs did not significantly predict maximum failure load $R^2=0.19$, $p=0.100$ (Figure 6.1A). The median T_2^* value also did not predict maximum failure load, $R^2=0.06$, $p=0.380$ (Figure 6.1B). Using volume with the median T_2^* value, the multiple linear regression model did not predict maximum failure load for the intact human ACL; $R^2=0.23$, $p=0.200$) (Figure 6.1C). Similar insignificant results were found for yield load and linear stiffness (Table 6.2).

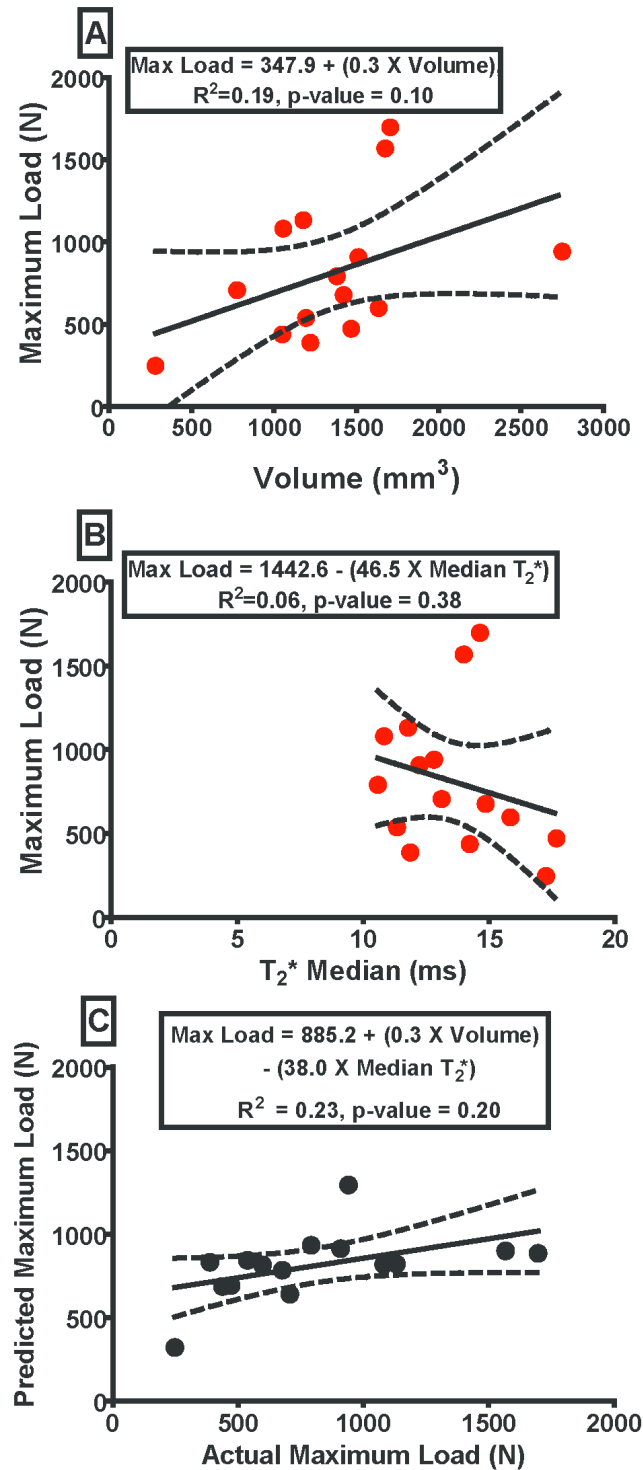


Figure 6.1: A) The ligament maximum load as a function of ligament volume and B) ligament maximum load as a function of ligament median T_2^* in linear regression models. C) Actual ligament maximum load versus predicted ligament maximum load determined using a multiple regression model as a function of the linear combination of ligament volume and median T_2^* . Dashed lines represent 95% confidence interval. Maximum load prediction equations for the intact ACLs as a function of volume (VOL), median T_2^* , and the linear combination of VOL and median T_2^* are inlaid in the graphs.

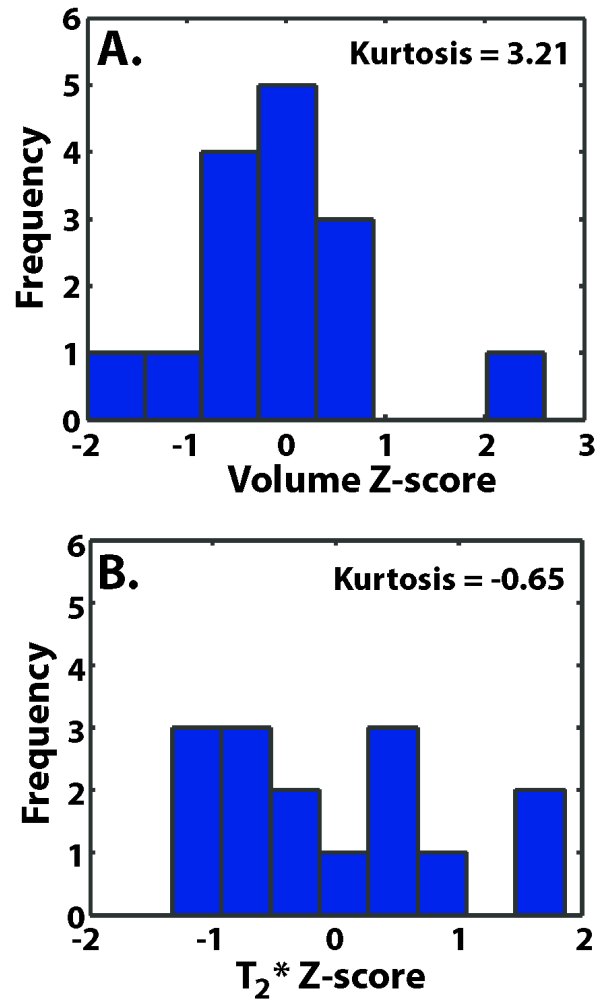


Figure 6.2: Histograms of human cadaveric intact ligament Z-scores of (A) volume and (B) median T_2^* .

	Max	Min	Median	25% CI	75% CI
Age (years)	76	24	54	42	62
Height (inches)	72	62	68	67	71
Weight (lbs)	200	87	170	125	180
BMI	33	13	23	19	27

Table 6.1: Human Cadaveric Demographics for 5 women and 10 male specimens.

6.3.2 Summary Statistics

The median (maximum–minimum) values for ligament volume, median T_2^* and maximum load were 1382.5 mm³ (2750.2-283.3 mm³), 13.1 ms (17.7-10.6 ms), and 706.8 N (1696.6-248.0 N), respectively. The 25 to 75% confidence intervals for ligament volume, median T_2^* , and maximum load, were 1087.6-1605.3 mm³, 11.8-14.8 ms, and 488.6-1046.0 N, respectively. The kurtosis values for ligament volume, median T_2^* , and maximum load were 3.2, -0.7, and 0.3, respectively (Figure 6.2, Table 6.3). Summary statistics for yield load and linear stiffness can be seen in Table 6.3.

Dependant Variable	Independent Variable	R ²	Regression p-value	Prediction Equation
Yield Load (N)	VOL	0.18	0.111	Yield (N) = 296.7 + (0.3 X VOL)
Yield Load (N)	Median T_2^*	0.05	0.437	Yield (N) = 1262.9 - (39.2 X T_2^* Median)
Yield Load (N)	VOL & Median T_2^*	0.21	0.238	Yield (N) = 738.4 + (0.3 X VOL) - (31.2 X T_2^* Median)
Stiffness (N/mm)	VOL	0.00	0.972	Stiff (mm/N) = 106.3 + (0.00 X VOL)
Stiffness (N/mm)	Median T_2^*	0.14	0.176	Stiff (mm/N) = 188.0 - (6.0 X T_2^* Median)
Stiffness (N/mm)	VOL & Median T_2^*	0.14	0.413	Stiff (mm/N) = 191.4 - (0.00 X VOL) - (6.0 X T_2^* Median)

Table 6.2: Summary of the yield load and linear stiffness equations for human cadaveric intact and ACLs as a function of volume (VOL), median T_2^* , and the linear combination of VOL and median T_2^* .

6.4 Discussion

The linear combination of ligament volume and median T_2^* value did not significantly predict the structural properties of the ACL in this human cadaveric model. This could be due to the naturally restricted variability of the data in the cadavers tested. The majority of the specimens fell in the volume range of 1087.6-1605.3 mm³ (25-75% confidence interval, Table 6.3) limiting the distribution of data to test the prediction. This was shown by the kurtosis value (3.2, Table 6.3), which indicated a high level of data clustering [1]. Z-scores of the volume measure also showed a restricted variability of the data set. Twelve of the 15 ligament specimens were less than 1 standard deviation away from the mean for the volume data set (Figure 6.2). Distributions of the T_2^* Z-scores also showed restricted variability, with 10 of the 15 ligaments being less than 1 standard deviation away from the mean for the distribution. These kurtosis and Z-score statistics indicate that the volume and T_2^* distributions were naturally restricted,

which can negatively affect the ability to detect a correlation when one exists and decrease the statistical power [7,12].

A previous study looking at porcine transected and reconstructed ligaments found that the linear combination of volume and T_2^* significantly predicted the healing ligament structural properties [2,3,16]. In this previous study, there were two different treatment groups of actively healing ligaments that resulted in a large range of evenly distributed volume and T_2^* data with little to no observed clustering [2]. This stands in contrast to the current human cadaveric study, where a small intact ligament volume was rare and the intact ligaments were not actively healing, leaving the volume and T_2^* distributions naturally restricted. Naturally restricted distributions of MR variables observed in the intact ACL of a porcine model were also found to have a similar insignificant ability to predict structural properties when compared to a proven prediction in a healing ACL porcine model [3](See Appendix C). While the MR variables did not significantly predict structural properties, there was evidence that high volume and short T_2^* times were associated with higher structural properties. When the specimens were split into a low failure and high failure group (<700 N, $n=7$ and >700 N, $n=8$, respectively) and tested for differences in volume and median T_2^* using a one way ANOVA, we found the specimens in the high failure group had significantly lower T_2^* values in comparison to the low failure group (12.5 vs 14.7 ms, $p\text{-value}=0.048$). Although the high failure group tended to have larger volumes (1505 vs 1182 mm³, $p\text{-value}= 0.261$), the difference was not significant.

Age negatively correlates to the structural properties of the intact cadaveric ACL; however, this correlation becomes insignificant in specimens over 48 years [15, 18]. Our cadaveric population was clustered around 50 years (25-75% confidence interval 42-62 years, Table 6.1), indicating our specimens fell in a similar age bracket where volume and T_2^* would not have the ability to predict structural properties. This further suggests the age of our test sample (clustered around 50) may not have been ideal to capture enough variability in the volume and T_2^* distributions to detect a correlation to structural properties.

Variable	Max	Min	Median	25% CI	75% CI	Kurtosis
Volume (mm ³)	2750.2	282.3	1382.5	1087.6	1605.3	3.2
Median T_2^* (ms)	17.7	10.6	13.1	11.8	14.8	-0.7
Max Load (N)	1696.6	248.0	706.8	488.6	1046.0	0.3
Yield Load (N)	1652.4	225.9	639.2	412.6	924.3	0.7
Stiffness (N/mm)	165.9	52.4	102.4	79.8	139.5	-0.9

Table 6.3: Summary statistics (Volume, Median T_2^* , Maximum Load, Yield Load, Stiffness) for the human cadaveric intact ACL.

Using the MR variables of volume and T_2^* (or SI) to detect ligament degeneration in terms of ligament strength are based on previous research showing that these same MR variables could predict ligament structural properties in animal models of ACL or graft healing. We assumed that the same trends with MR variables observed during the ligament healing process [2,3,16] would be observed in reverse order with a degenerating ligament. While T_2^* has been used to detect degeneration in the meniscus [17], there may be other factors specifically associated with the ligament degenerative process. Unlike the healing or regenerative process, these factors may involve characteristics besides graft size and quality. For example, osteophytes could induce damage to the ligament surface, which could influence the failure properties. Additionally, while the same trends were observed with volume and T_2^* in the current human cadaveric prediction compared to healing porcine ligaments (larger volume and shorter T_2^* times were associated with higher structural properties [2], there could be an inherent difference with the macro anatomy of the intact ligaments that lead to the insignificant findings reported here. For instance the collagen fibers of the intact ACL twist in the intra-articular space and may cause volume-averaging artifacts that could confound the signal intensity [11]. Further research will be needed to clarify if these additional factors may confound the results.

There were limitations to this study. To limit concerns over varying temperature effects across the samples [13], all knees were thawed to room temperature to standardize temperature for MR imaging. Additionally, while a carefully controlled freeze thaw cycle does not affect structural properties of ligaments [19], and our protocol controlled the number of these cycles, there is no certainty in specimen handling (number of freeze thaw cycles or delay between death and freezing) prior to receiving them. Two of the younger specimens had higher than expected T_2^* values and it is possible that unique circumstances of death or decay processes could have confounded these measurements. Finally, the availability and distribution of this data set may have limited our capacity to predict structural properties. With the current sample size ($n=15$) and considering the two independent variables in the regression model, the study was 0.80 powered to detect a multiple R^2 of 0.45 or higher [9]. Adding additional cadaveric specimens at younger and older ages may help to better distribute the ligament volume, median T_2^* and biomechanical performance; and could potential help detect a relationship. Possibly 30 or more total specimens, divided evenly between a lower, middle and later age ranges, may have helped with prediction strength [15,18]. Unfortunately, younger cadaveric knees are not easily obtained in high quantities for analysis.

Using a set of human cadaveric knees, we were unable to relate biomechanical outcome to MR measures of volume and T_2^* obtained *in situ*. Naturally restricted distributions of ligament volume and T_2^* were likely

the cause of the insignificant findings. Further research is necessary to understand the relationship of MR variables and ligament degeneration in a human cadaveric model. This study failed to find a significant prediction of human cadaveric biomechanical outcome using volume and T_2^* . Further research geared toward capturing younger cadaveric specimens may be valuable for creating an MR-based approach to longitudinally assess changes in cruciate ligament degradation and to assess cruciate integrity when determining indications for prosthesis type with TKA procedures.

6.5 Conflicts of interest

None.

6.6 Acknowledgements

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Chapter 7

MRI Volume and Signal Intensity of the ACL Graft Predicts Clinical, Functional and Patient Oriented Outcome Measures Following ACL Reconstruction

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Abstract

Background: Clinical, functional and patient-oriented outcomes are commonly used to evaluate the efficacy of treatments following ACL injury; however, these evaluation techniques do not directly measure the biomechanical changes that occur with healing.

Purpose: To determine if the magnetic resonance (MR) image-derived parameters of graft volume and signal intensity (SI), which have been used to predict the biomechanical (i.e., structural properties) of the graft in animal models, correlate with commonly used clinical (anteroposterior (AP) knee laxity), functional (1-leg hop) and patient-oriented outcome measures (KOOS) in patients 3- and 5-years after ACL reconstruction.

Study Design: Descriptive Laboratory Study

Methods: Using a subset of participants enrolled in an ongoing ACL reconstruction clinical trial, AP knee laxity, 1-legged hop test, and KOOS were assessed at 3- and 5-year follow-up. 3-D T₁-weighted MR images were collected at each visit. Both the volume and median SI of the healing graft were determined and used as predictors in a multiple regression linear model to predict the traditional outcome measures.

Results: Graft volume combined with median SI in a multiple linear regression model predicted 1-legged hop test at both the 3-year and 5-year follow-up visits ($R^2=0.40$, $p=0.008$ and $R^2=0.62$, $p=0.003$, respectively). Similar results were found with 5-year follow up for the KOOS quality of life ($R^2=0.49$, $p=0.012$), sport/function ($R^2=0.37$, $p=0.048$), pain ($R^2=0.46$, $p=0.017$) and symptoms ($R^2=0.45$, $p=0.021$) sub-scores, though these variables were not significant at 3-years. The multiple linear regression model for AP knee laxity at 5-year follow-up approached significance ($R^2=0.36$, $p=0.088$).

Conclusion: The MR parameters (volume and median SI) used to predict *ex vivo* biomechanical properties of the graft in an animal model have the ability to predict clinical or *in vivo* outcome measures in patients at 3- and 5-year follow-up.

Clinical Relevance: Results from this study may enhance clinical evaluation of graft health by relating the MR parameters of volume and median SI to traditional outcome measures and could potentially aid researchers in determining the appropriate timing for athletes to return to sport.

Keywords: MRI, ACL, clinical assessment, patient outcome, biomechanics

7.1 Introduction

Current clinical, functional and patient-oriented outcome assessments for evaluating the success of anterior cruciate ligament (ACL) treatments include hop testing, knee arthrometry and patient-oriented outcome questionnaires. These assessment techniques have been useful in many clinical studies as a standardized way to evaluate the success of the treatment.[11] However, because these evaluation techniques are an indirect measure of graft health or integrity, they require large numbers of patients to provide sufficient power and to detect differences between treatment groups. The lack of sensitive outcome measures for clinical studies may be one reason why no improvements have been found in many clinical trials comparing outcomes of different ACL reconstruction techniques (e.g., comparisons of graft type,[21] graft position,[1] rehabilitation,[2] or graft tension [11]). Furthermore, most of these knee specific outcome measures may not be sensitive enough to detect biomechanical changes in the graft itself during healing. As a result *ex vivo* animal models are frequently used to determine the biomechanics of a reconstructed graft to directly evaluate graft healing.[12,23] However, *ex vivo* approaches require destructive testing and are not suitable for longitudinal *in vivo* use. This limitation makes a reliable, quantitative, *in vivo*, method for determining the biomechanical performance of a graft during healing highly desirable in both research and clinical settings.

Magnetic resonance (MR)-derived measures of volume [13,15] and signal intensity (SI) [3,34] have been found to be independent predictors of graft or ligament failure properties. More specifically, the linear combination of graft volume and median SI has been found to correlate to the biomechanical properties measured via *ex vivo* mechanical testing and offers a more complete evaluation of ligament or graft integrity than either MR-derived variable alone.[3,34] While a method that directly relates these MR-derived parameters to the graft biomechanical properties is an important finding, determining the relationship between a graft's MR parameters and a patient's knee specific traditional outcomes will offer a graft specific assessment to complement the existing clinical evaluation tools.

The objective of this study was to determine if the MR parameters (volume and SI), which have been used to predict the biomechanical properties of the graft,[3,4] will predict clinical (AP knee laxity), functional (1-leg hop for distance) and patient-oriented outcome measures (Knee Osteoarthritis Outcome Scores) at 3- and 5-years after ACL reconstruction.[11] We hypothesize that the MR-derived parameters of graft volume and median signal intensity will significantly predict these clinical, functional and patient-oriented outcomes in an ACL reconstructed cohort. Results from this study will provide a translational link between *ex vivo* animal model data of graft healing and commonly used clinical assessment tools. Furthermore,

these data could help in the development of a more objective test to determine the appropriateness of timing for athletes to return to sports.

7.2 Methods

7.2.1 Patient Populations

A subset of participants enrolled in an ongoing Institutional Review Board (IRB) approved study, investigating the effects of initial graft tension on long-term outcome of ACL reconstruction with autograft, was used for this analysis (NCT00434837).[11] Patients received either a high-tension or low-tension graft following an isolated unilateral ACL injury as previously described.[11] Postoperatively, all patients followed a standardized rehabilitation program designed to get them back to sport within 6 months. The primary study results at 3-year follow-up found no significant differences in the clinical, functional and patient-oriented outcome measures between the high- and low-tension treatment groups.[11] Subsequently, all patients with complete traditional outcomes and MR scans were pooled for this analysis. Of the 90 patients enrolled in the original clinical study,[11] 64 completed an onsite visit to obtain the clinical and functional outcomes at the three year follow-up. Of those 64, 50 MR images were collected. Of the 50 patients with complete traditional outcomes and MR scans, 29 of those scans were confounded by metal artifact in the immediate area of the graft and were omitted as it was not possible to obtain accurate volume and SI measurements. The study group at 3 years (23 total; 10 men, 13 women) had a mean age of 23 ± 9 years at time of surgery. Seventeen patients received bone–patellar tendon–bone autograft obtained from the central third of the ipsilateral patellar tendon and 6 received a 4-stranded autograft created from the semitendinosus and gracilis tendons. Ten received a low-tension graft and 13 received a high-tension graft. Of the 23 patients with complete traditional outcomes and MR images obtained at 3 years, 17 returned for an onsite follow-up at 5 years. Of these 17, 16 underwent MR imaging and none were excluded due to artifact. At 5 years the study group (16 total; 6 men, 10 women) had a mean age of 24 ± 10 years at time of surgery. Eleven received bone–patellar tendon–bone autografts and 5 received 4-stranded autografts. Seven received a low-tension graft and 9 received a high-tension graft.

7.2.2 Traditional Outcomes

Clinical, functional and patient-oriented outcomes were used to assess overall patient knee function and patient outcome and to establish the relationships with the MR parameters of graft healing.

7.2.2.1 Clinical Outcome

AP knee laxity values for both knees were measured using an arthrometer (KT-1000: MEDmetric Corp, San Diego, CA) at 3- and 5-year follow-up. Anterior-directed shear loads were applied in succession to find the neutral position of the knee. Three manual maximum tests were then performed and the displacement readings between -90 Newtons of posterior shear load and the manual maximum anterior shear load were averaged. The AP knee laxity score was reported as the difference in displacement between the injured knee and the uninjured contra-lateral knee (APlaxity difference). One examiner, with more than 6 years of experience, performed all of the arthrometer measurements.

7.2.2.2 Functional Outcome

At each follow-up visit, patients performed the 1-legged hop test for distance independently 3 times, and the trials were averaged.[29] The mean hop distance of the injured knee was normalized to that of the uninjured contralateral knee to determine the patient's hop score (hop%).

7.2.2.3 Patient-Oriented Outcome

The KOOS[30] was implemented to assess patient-oriented outcomes of the cohort at both time points. The KOOS evaluates 5 domains: knee-related quality of life (KOOS-qol), sports and recreation function (KOOS-spt), pain (KOOS-pain), symptoms (KOOS-sym), and activities of daily living (KOOS-adl).[30]

7.2.3 MRI Ligament Outcomes

The MR parameters (volume and median SI), which have been used to predict the biomechanical properties of the graft,[3,4] were used to quantify graft integrity. All MR images were acquired 3- and 5-years

post-operatively using a surface knee coil on the same 3T scanner (Siemens TIM Trio, Erlangen, Germany) using standardized protocol and acquisition parameters. A 3-D T_1 -weighted FLASH sequence (TR/TE/FA, 20/7.6/ 12°; FOV, 160 mm; matrix 512X512, slice thickness/gap, 1.5mm/0; avg 1; bandwidth, 130) was used. Scans with confounding metal artifact due to magnetic susceptibility effects in the immediate proximity to the graft were omitted from analysis. These artifacts were most likely generated from the metallic drill bits used for tunnel placement during the reconstruction procedure. Some patients did receive a metallic fixation screw but in all cases the screw was sufficiently far from the intra-articular space to avoid direct issues with artifact and the ACL. Each ACL graft was then manually segmented from the MR image stacks and 3-D models of the graft were created using commercially available software (Mimics 16.0; Materialize, Ann Arbor, MI).[3] Summing the total number of ACL graft voxels provided an estimate of the whole graft volume (6.94 voxels equaled one mm³). The median graft SI (grayscale value) was calculated for each patient and was normalized to the subject-specific SI of femoral cortical bone to minimize inter-scan variability.[3,31] All graft segmentations, for both 3- and 5-year scans, were done by one examiner with more than 5 years of experience. The images were randomly processed within a six month time period. Prior to beginning the study, the intra-examiner reliability was tested using seven repeated scans of a human cadaveric knee and four repeated scan of a porcine reconstructed knee and a coefficient of variation of less than 5.8% was observed for both volume and signal intensity measures.

7.2.4 Data Analysis

Because median graft SI values were not normally distributed, values were log transformed (Base 2) prior to analysis as previously reported.[3] Both volume and median graft SI were included as independent variables in a first order multiple linear regression model to predict the traditional outcome measures (i.e. knee laxity (APLaxity difference)), hop score (hop%), knee-related quality of life (KOOS-qol), sports and recreation function (KOOS-spt), pain (KOOS-pain), symptoms (KOOS-sym), and activities of daily living (KOOS-adl)). In addition to reporting the individual slope coefficients and the significance of the two predictors (volume and SI), the model R^2 is also presented as a measure of overall model performance.[9,24] Regression diagnostics based on residual plots were used to evaluate the appropriateness of the linear model.[9] Randomness of residuals was accepted for all variables except KOOS-adl, which was due to the limited range of patient scores (most patients had perfect KOOS-adl scores). Additionally, multi-collinearity between volume and SI in the multiple regressions was assessed using the variance

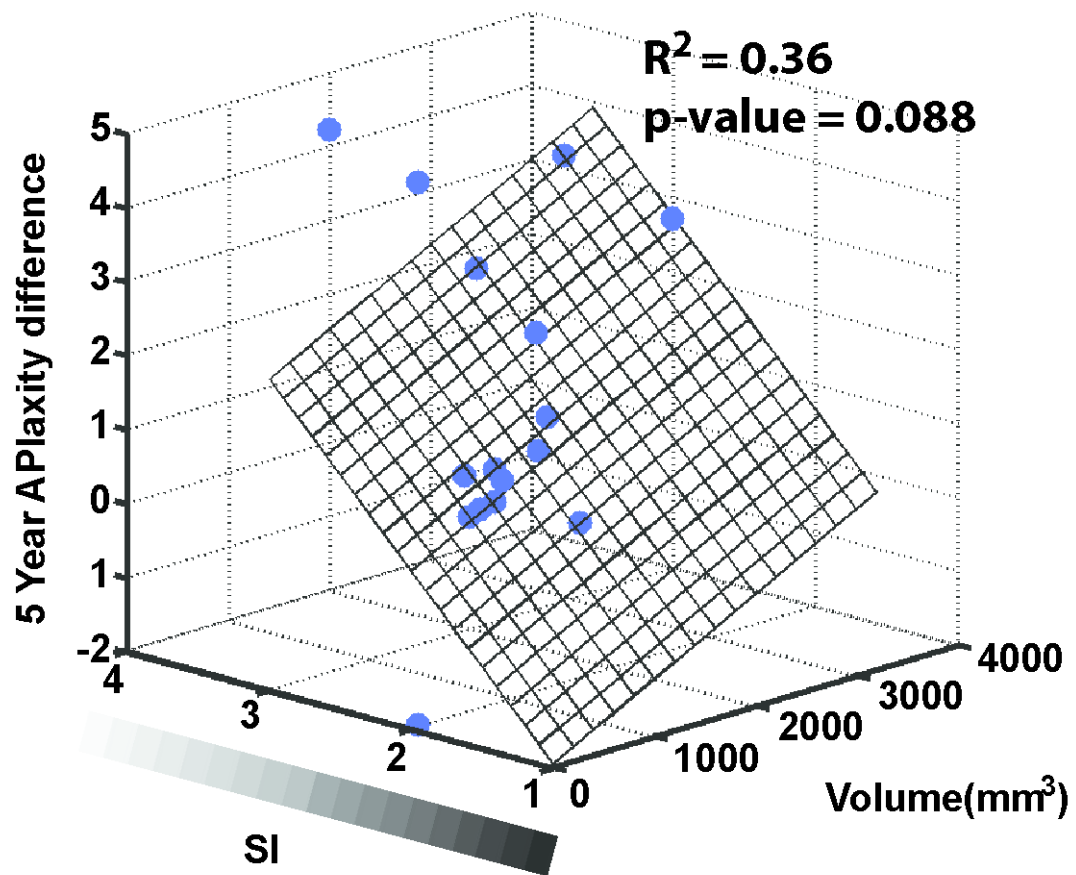


Figure 7.1: The patient graft prediction plane for knee APlaxity difference as a function of graft volume and median SI at 5-year follow-up ($R^2 = 0.36$, $p=0.088$). The grafts with the higher volume and lower SI tended to have lower APlaxity difference scores (injured minus contra-lateral).

inflation factor (VIF) at both 3- and 5-year time points. VIF values were 1.01 for 3-year and 1.11 for 5-year time points and were well below the recommended limit of 10, as stated in the literature.[20,25] A VIF of 1 indicates a stable regression. Statistical analyses were performed using SAS statistical software (SAS Institute, Cary, NC).

7.3 Results

7.3.1 Clinical Outcomes

Graft volume combined with median graft SI in a multiple linear regression model did not predict APlaxity difference at 3-year follow-up (Table 7.1). Likewise, the combination of volume and median SI did not predict APlaxity difference at 5-year follow up; however, it did approach significance ($R^2=0.36$, $p=0.088$) (Figure 7.1, Table 7.1).

7.3.2 Functional Outcome

Volume combined with median graft SI in a multiple linear regression model predicted hop% at 3-year follow-up ($R^2=0.40$, $p=0.008$) (Figure 7.2a, Table 7.1). The combination of volume and SI also predicted hop% at 5-year follow up ($R^2=0.62$, $p=0.003$) (Figure 7.2b, Table 7.1). (Figure 7.4).

7.3.3 Patient-Oriented Outcome

At 3-year follow-up, volume combined with median graft SI in a multiple linear regression model did not predict KOOS-qol, KOOS-spt, KOOS-pain, KOOS-sym, KOOS-adl (Table 7.1). However, at 5-year follow-up, the combination of volume and SI predicted KOOS-qol ($R^2=0.49$, $p=0.012$) (Figure 7.3), KOOS-spt ($R^2=0.37$, $p=0.048$), KOOS-pain ($R^2=0.46$, $p=0.017$), KOOS-sym ($R^2=0.45$, $p=0.021$) (Table 7.1). At 5-year follow-up, the linear combination of volume and median SI did not predict KOOS-adl (Table 7.1).

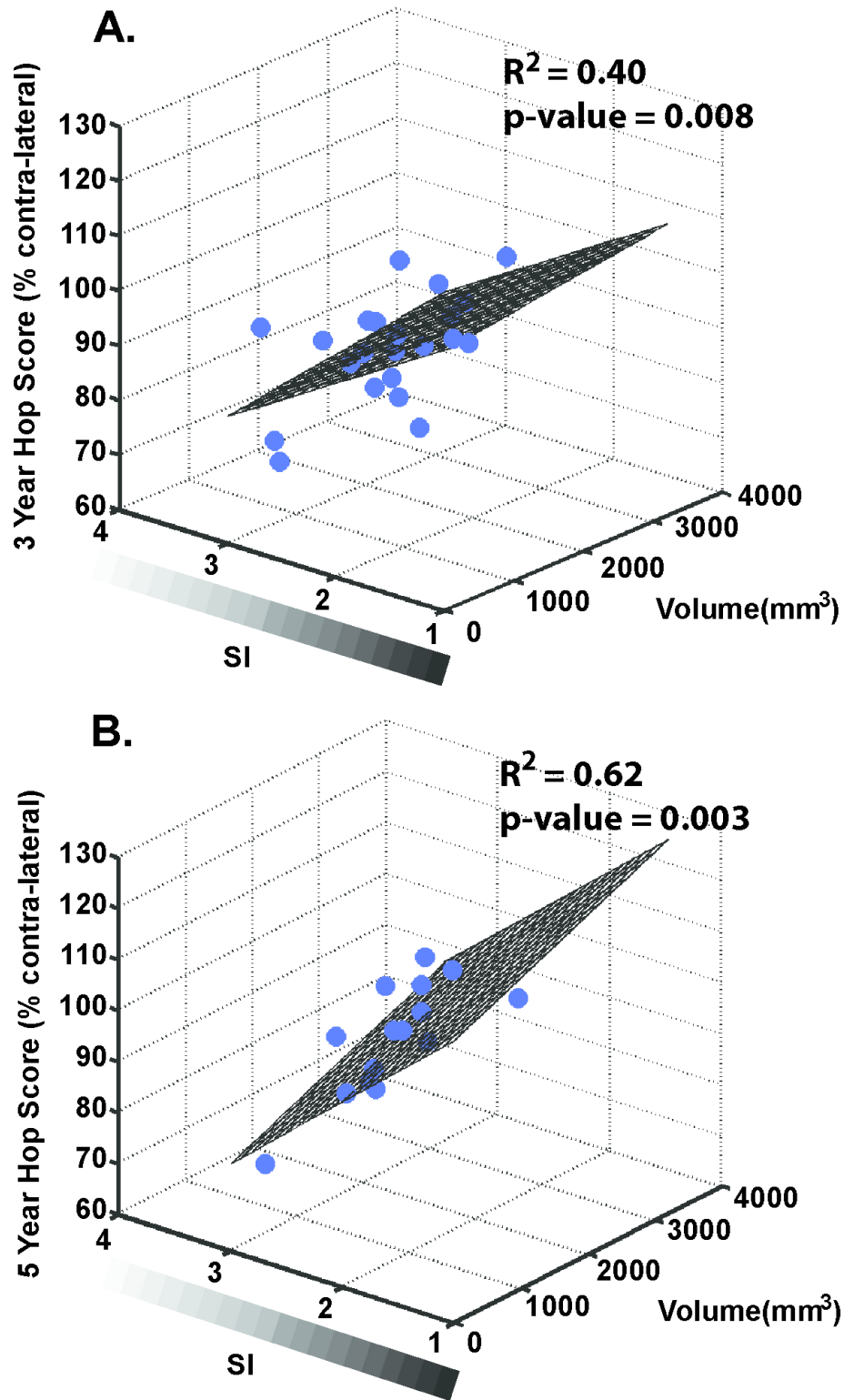


Figure 7.2: The patient prediction planes for hop score as a function of graft volume and median SI at A) 3-year follow-up ($R^2 = 0.40$, $p=0.008$) and B) 5-year follow-up ($R^2 = 0.62$, $p=0.003$). The grafts with the higher volume and lower SI tended to have higher hop scores (% injured vs contra-lateral).

7.4 Discussion

Knee arthrometry, hop testing, and patient-oriented outcome questionnaires have been useful for many clinical studies as a standardized way to evaluate overall patient knee outcome following ACL treatment;[11] however, these evaluation techniques are knee specific measures of joint and patient health but may lack the sensitivity to determine the biomechanical properties of the graft. A more specific measure of graft integrity would therefore be a useful complement to the already existing set of treatment evaluation tools. Using patients from an ongoing ACL reconstruction study[11] we were able to show that the same MR parameters (volume and median SI) used to directly predict *ex vivo* biomechanical properties of the graft in an animal model[3,4] have the ability to predict overall knee health in terms of functional and patient-oriented outcome measures in patients.

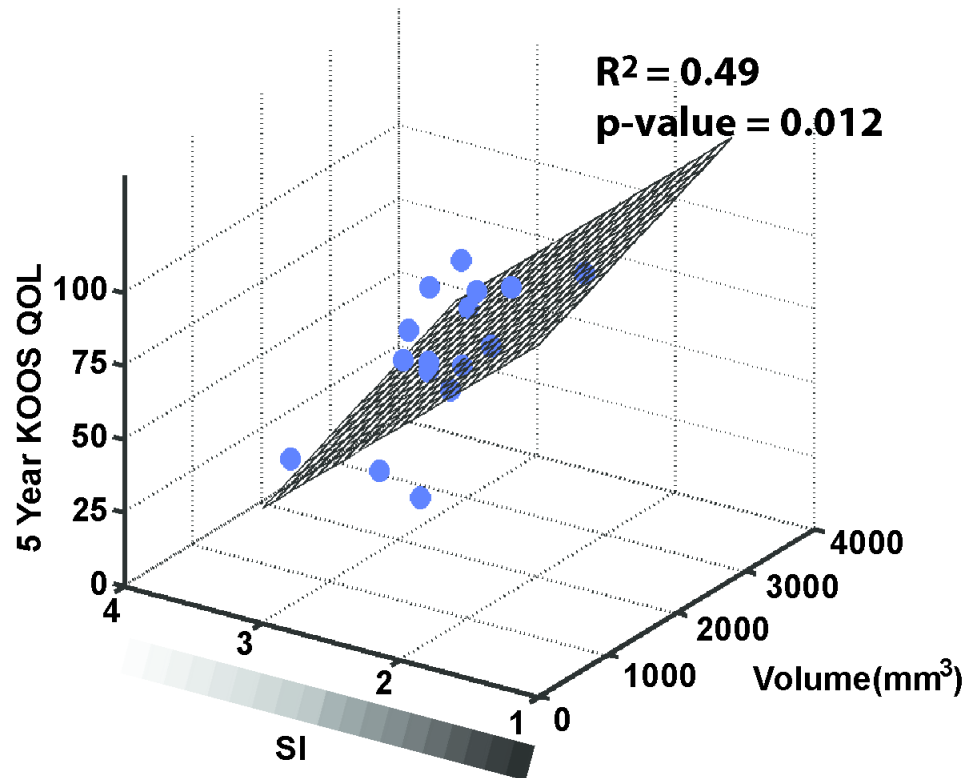


Figure 7.3: The patient prediction plane for KOOS-qol sub-score, as a function of graft volume and median SI at 5-year follow-up ($R^2 = 0.49$, $p=0.012$). The grafts with the higher volume and lower SI tended to have higher KOOS-qol sub-scores (100 being perfect knee function). Similar plots were found for the KOOS-spt, KOOS-pain and the KOOS-sym 5-year follow-up prediction models.

In general, for traditional outcomes at 5-year follow-up, larger grafts with lower median SI values were associated with better knee performance and surgical outcome (Figure 7.4). For the functional outcome at 5-year follow-up, patients with higher hop% (higher percent score reflects better knee function),[28]

Traditional Outcome	Dependent Variable	Independent Variable	3-year				5-year			
			Coefficient	p-value	Model R ²	Model p-value	Coefficient	p-value	Model R ²	Model p-value
Clinical	<i>AP Laxity difference</i>	<i>Intercept</i>	0.821		0.12	0.294	-4.0		0.36	0.088
		<i>Volume</i>	-0.0012	0.213			0.0007	0.304		
		<i>SI</i>	1.067	0.426			2.1	0.099		
Functional	<i>1-Leg Hop</i>	<i>Intercept</i>	135.5		0.40	0.008*	134.5		0.62	0.003*
		<i>Volume</i>	-0.002	0.652			0.006	0.050*		
		<i>SI</i>	-17.4	0.002*			-19.1	<0.001*		
Patient-Oriented	<i>KOOS-qol</i>	<i>Intercept</i>	110.5		0.09	0.396	160.4		0.49	0.012*
		<i>Volume</i>	0.005	0.580			0.011	0.137		
		<i>SI</i>	-17.8	0.245			-40.4	0.004*		
	<i>KOOS-spt</i>	<i>Intercept</i>	126.5		0.14	0.232	122.2		0.37	0.048*
		<i>Volume</i>	-0.003	0.592			0.013	0.054		
		<i>SI</i>	-15.9	0.097			-23.8	0.033*		
	<i>KOOS-pain</i>	<i>Intercept</i>	101.1		0.01	0.842	123.6		0.46	0.017*
		<i>Volume</i>	-0.001	0.739			0.004	0.163		
		<i>SI</i>	-3.5	0.609			-15.4	0.006*		
	<i>KOOS-sym</i>	<i>Intercept</i>	97.8		0.03	0.770	136.6		0.45	0.021*
		<i>Volume</i>	0	0.997			0.006	0.211		
		<i>SI</i>	-5.9	0.479			-26.1	0.007*		
	<i>KOOS-adi</i>	<i>Intercept</i>	101.4		0.02	0.808	110.3		0.14	0.372
		<i>Volume</i>	0.001	0.823			0.004	0.322		
		<i>SI</i>	-3.2	0.569			-9.2	0.213		

Table 7.1: Summary of the patient outcome prediction equations for both the 3- and 5-year follow up as a function of graft volume and SI in terms of median grayscale value (log base 2 transform). Stars indicate significance.

tended to have larger grafts with lower median SI (Figure 7.2). Similarly, for the KOOS-spt, KOOS-pain, KOOS-qol, and KOOS-sym sub-scores at 5-year follow-up, patients with larger graft volumes and lower SI had higher sub-scores (higher scores indicate better knee function) (Figure 7.3).[6] At 5-year follow-up, patients with higher APlaxity difference scores (i.e., more surgical knee laxity than the contralateral control),[5] while not significant tended to have grafts with smaller volume and higher median SI (Figure 7.1). Previous research has shown larger graft or ligament volume [3,13,15] and lower graft or ligament SI [3,34] are correlated to higher strength or biomechanical properties. These results show that MR parameters that relate to graft biomechanical performance are also predictive of overall patient knee health and ACL reconstruction surgical outcomes.

At 3-year follow-up, the MR variables of volume and median SI were unable to predict clinical (APlaxity difference) and patient-oriented outcomes (KOOS sub-scores). These non-significant predictions at the 3-year follow-up paired with lower observed standard deviations in both volume at the 3-year compared to the 5-year follow-up (Volume: 618 vs 711 mm³; SI: 0.39 vs 0.43, respectively) suggest that there may not be enough variability [7,18] in patient graft volume and SI to predict traditional outcomes at this earlier time point. However, at 3-year follow-up the prediction for hop% was significant. Additionally, we saw an increase in prediction between the 3- and 5-year hop% data as indicated by increasing R² values (3-year R²=0.40, 5-year R²=0.62). Considering this increase in R² and the significant 5-year prediction of the KOOS sub-scores (KOOS-spt, KOOS-pain, KOOS-qol, and KOOS-sym sub scores), these results suggest that patient graft volume and SI are more heterogeneous at the 5-year follow-up time, and may indicate that the graft is still remodeling at 3 years. The linear combination of graft volume and median graft SI were unable to predict the KOOS-adl sub-score at either 3- or 5-year follow-up. These results support previous research showing that the KOOS-adl sub-score has been reported to be the least indicative of patient surgical outcome.[6,11]

The relative contribution of the independent variables of graft volume and median graft SI were assessed with individual p-values in the linear regression.[19] Median graft SI significantly contributed to the predictions for all 5-year traditional outcomes (all $p \leq 0.048$, Table 7.1), except the APlaxity difference and KOOS-adl. SI was also significant at 3-year follow up for the hop test. Median graft or ligament SI has shown to be a significant predictor of *ex vivo* biomechanical properties in an animal model,[3,34] further indicating graft integrity as represented by median SI may reflect surgical outcomes. Graft volume was only a significant contributor for the hop test at 5-year follow up and approached significance for the KOOS sport 5-year follow up. Despite being a significant predictor in *ex vivo* models,[3,13,15] graft volume may not be as strong of a predictor in this study due to variations in patient graft type.

ANOVA's were used to test for differences between patient graft type for both volume and SI. No significant differences were found with graft volume or SI between graft types. Additionally, ANOVA's were used to test for differences between the high tension and low tension group for both volume and SI. No significant differences were found with volume and SI between the tension groups. This finding reflects the lack of differences in the tension groups for the original clinical study.[11]

SI has been used in prior clinical studies to evaluate ACL graft health and maturation following ACL reconstruction surgery.[10,16,22,27,32] More specifically, it was found that four years after ACL reconstruction semi-quantitative clinician graded scores based on graft SI appearance were unable to predict knee laxity or the International Knee Documentation Committee clinical outcome score. The lack of correlation found in this prior study could be due to the semi-quantitative nature of the clinician-based grading system,[17,27,32] which may lack the specificity needed to detect subtle differences in graft integrity. Furthermore, the authors cited a study selection bias[32] where patient recruitment was done after ACL reconstruction surgery, which may have limited the patient population to those with positive surgical outcomes, limiting the variability in the treatment outcomes.

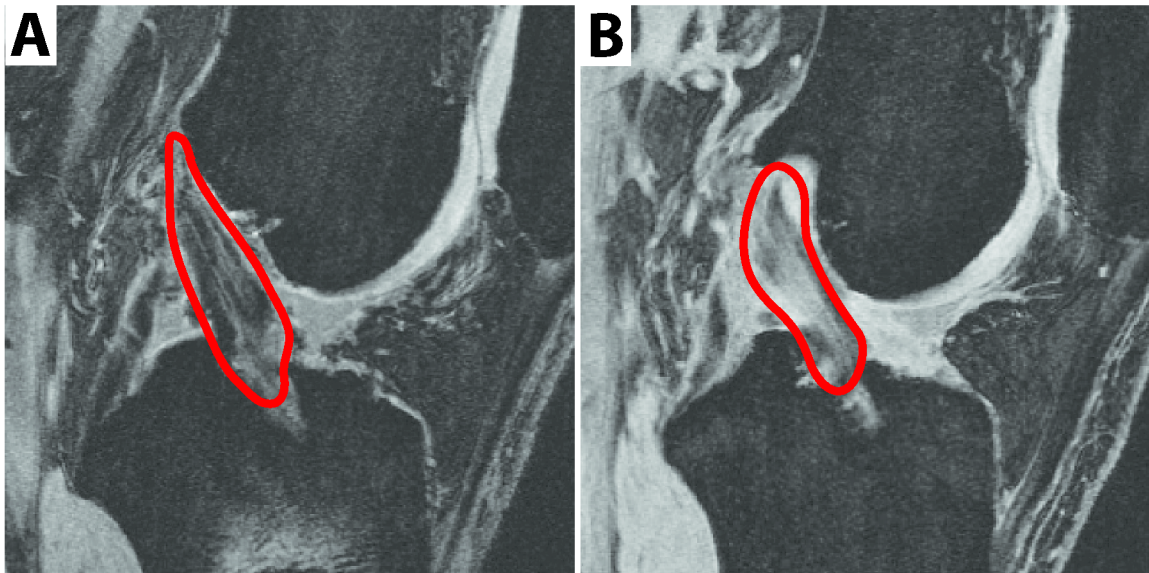


Figure 7.4: Example A) low and B) high SI for patient grafts on one sagittal slice of the MR image stack.

This study was limited by the use of the SI variable, which can vary depending on scanner hardware and MRI acquisition parameters.[8] To address this concern we used the same MR imaging parameters and manufacturer (Seimens, 3T Trio) throughout the study. Furthermore, we normalized the graft SI values to that of cortical bone within each image to minimize concerns of variability between scan sessions.[3,31] Another limitation was the metal artifact identified in some of the follow-up MR scans. To address this

limitation, we omitted scans with confounding metal artifact. However, metal artifact is not uncommon in MR images of patients' knees following ACL reconstruction.[14,33] The problem could be minimized in part with the use of non-metallic fixation screws and flushing the joint to flush residual debris prior to closure. It was assumed the MR parameters (volume and median SI) used to directly predict *ex vivo* biomechanical properties of the graft in an animal model[3,4] would directly translate to a human clinical population. However, *ex vivo* failure testing of the graft is not possible in a clinical population. Therefore, this study was built on the research performed in a porcine model.[26] For this study, we assumed that graft volume and SI were the only MR variables affecting patient outcome. However, it is possible that MR analyses to directly quantify fiber alignment and orientation could bolster the predictions of patient outcomes. Also, finding that graft volume and SI were predictive of traditional outcomes does not imply causality. It is possible that poor patient surgical outcome caused graft changes that are then detected through MRI rather than graft MR appearance affecting patient traditional outcomes. Further research would be necessary to clarify this point. Finally, at this point it is unknown how time would affect the relationships between the independent variables (volume, SI) and traditional outcomes, as 3- and 5-years following ACL reconstruction may not be the ideal time to evaluate the healing process. Future studies will focus on earlier time points when the graft is expected to be actively remodeling. If the relationship between volume and SI and traditional outcomes are found to hold at earlier time points, it could allow clinicians to determine graft specific health to determine if the graft is healed enough to return to sport.

Despite these limitations, the results from this study provide a valuable translational link between *ex vivo* animal model data and patient data collected in a clinical context. With further development these data could be used as a more objective test to determine the appropriateness of timing for the athletes return to sports.

7.5 Conflicts of interest

None.

7.6 Acknowledgements

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Chapter 8

Conclusions, Related Studies, and Future Directions

8.1 Conclusions

The studies and data presented in this dissertation describe and support a non-invasive MR method for predicting the structural properties or histological *outcomes* of an ACL graft or ligament. The long-term objective of this work was to establish a non-invasive MR method that would allow researchers to document functional healing longitudinally within an animal model specimen in pre-clinical studies. This prediction method will be advantageous for evaluating ligament or graft health non-invasively, thus removing the need for euthanasia and mechanical testing at early time points and reducing the number of animals and resources required for a study. Furthermore, these findings may have implications as a surrogate outcome measure in clinical studies for documenting temporal changes within-patients and as a more quantitative method for guiding rehabilitation and determining when a patient is ready to return to sport. The specific aims of the chapters presented in this dissertation were to design (Specific Aim 1) and refine (Specific Aim 2, Specific Aim 3, Specific Aim 4) a universal MR imaging method for predicting the structural properties or histological outcomes of a healing ligament or graft in a porcine model, translate the method to the degeneration process in the intact human ACL (Specific Aim 5), and correlate the MRI measures with clinical, functional, and patient oriented outcome measures for a healing graft following treatment (Specific Aim 6). The contents of Chapter 1 detail the background and significance of the research presented, and specifies the purpose and hypotheses of the specific aims.

8.1.1 Specific Aim 1

Develop a method to predict the structural properties of a healing ligament (ACL primary repair) and graft (ACL reconstruction) in a porcine model using the MRI derived parameters of volume and signal intensity (SI).

The first aim was addressed with the study presented in Chapter 2. Our objective with this study was to determine ligament volume and signal intensity (SI) of reconstructed and bio-enhanced repaired ACLs in a porcine model. We then evaluated the relationship of these MR variables to ligament structural properties by using an *ex vivo* tensile testing protocol. We hypothesized that intra-articular graft or ligament volumes and SI values, in terms of median grayscale, would be significant predictors of the structural properties of the reconstructed ligament and ACL repairs after 15 weeks and 52 weeks of healing. Additionally, we hypothesized that grafts and ligaments at 15 weeks would have significantly higher SI values than at 52 weeks.

We found volume significantly predicted the structural properties (maximum load, yield load, linear stiffness) of the ligaments and grafts. Likewise, SI values significantly predicted the structural properties of the ligaments and grafts. By combining these two parameters in a multiple regression model we saw an improved ability to predict graft or ligament structural properties, indicating the combination of these variables offers a more complete evaluation of graft integrity than either variable alone. Additionally, we found that grafts and ligaments at the 15 week time point had a significantly higher SI value than ligaments at 52 weeks. This observed difference between the 15 week and 52 week groups could indicate healing and maturation of the graft or ligament on a tissue level. In conclusion, volume and SI from T_2^* weighted MRI images are predictive of structural properties of the healing ligament or graft in a porcine model. This study provides a critical step in the development of a non-invasive method to predict the structural properties of the healing ACL graft or repair.

The SI variable used in this study can vary depending on MR imaging parameters. We normalized the grayscale values to that of cortical bone within each image to minimize this concern. However, the use of SI as an outcome measure is limited by this dependence on image acquisition parameters as well as, scanner manufacturer and hardware, rendering the predictions to be protocol, magnet, and hence, institution specific. One way to standardize or normalize MR results between scanners is to use relaxation time variables, such as T_2 and T_2^* . These variables are inherent tissue properties that reflect specific tissue characteristics, and are much less sensitive to image acquisition parameters than conventional signal intensity data. T_2^* relaxation time is an MR parameter that has been shown to correlate with the level of tissue organization, and is thus well suited for imaging highly organized collagenous structures, such as ligaments. Specifically, the water interactions with collagen restrict its mobility (residual dipolar coupling, susceptibility anisotropy, and bulk magnetic susceptibility) and this constraint causes rapid relaxation of the MR signal following RF excitation. This effect accounts for the short T_2^* in ligaments and other structures with highly organized ultrastructure and an abundance of collagen.[26] This suggests that variations in collagen integrity can be visualized by quantifying T_2^* . Thus, T_2^* relaxation time could provide a more universal prediction model of the structural properties of a healing ligament that would be applicable across scanners of the same strength and between institutions.

8.1.2 Specific Aim 2

Develop and test a method to collect T_2^ relaxation time as a universal MR outcome that is tailored to imaging a healing ligament or graft. A secondary aim of this study was to compare the ability of*

the T_2^ relaxation time parameter to predict structural properties to that of the previous signal intensity parameter (Specific Aim 1), with the intent of determining the relative uncertainty of the measures.*

The second aim of this thesis was addressed in Chapter 3. To address this aim we used a multi-echo imaging protocol for T_2^* determination, in order to collect the expected range of T_2^* values for a healing ligament. We collected MR images of a sub-set of the bio-enhanced long-term animals from Specific Aim 1 and determined ligament volume, SI and T_2^* values. For these animals, surgical ACL transection followed by no treatment (i.e., natural healing) or bio-enhanced ACL repair was performed. After 52 weeks of healing the limbs were harvested and MR images of the knees were collected. From these images, ligament volumes and T_2^* maps were established. The structural properties of the ligaments were also determined via *ex vivo* tensile testing. Using the T_2^* histogram profile, each ligament voxel was binned based on its T_2^* value into four discrete tissue sub-volumes defined by specific T_2^* intervals. We hypothesized that a multiple regression model based on ligament volume and its corresponding T_2^* values would provide a noninvasive predictor of the ligament's structural properties after 52 weeks of healing. We also hypothesized that the coefficients of determination would be greater and that the standard errors would be less when using the T_2^* prediction method compared to the signal intensity method.

We found the linear combination of the ligament sub-volumes defined by increasing T_2^* intervals significantly predicted structural properties of a healing porcine ACL at 52 weeks post-operatively. The T_2^* model was found to be comparable to the original signal intensity prediction model and offered similar certainty as defined by R^2 and p-values when determining structural properties.

Biomechanical performance in terms of structural properties offers an invaluable quantifiable assessment of ligament healing on a gross scale. However, microscopic evidence of healing, addressed via histology, is another important means to evaluate healing in a ligament. Thus it would be ideal if histological assessment of healing could also be predicted non-invasively with MR determined variables. More specifically, determining what histological factors contribute to short T_2^* times could be valuable in the research and clinical settings.

8.1.3 Specific Aim 3

Determine if T_2^ relaxation time can be used to predict semi-quantitative histological outcomes of the healing ACL.*

Addressed in Chapter 4 of this thesis, this aim was designed to test if the same MR variables (T_2^* and Volume) that could predict structural properties in the healing ACL can also be used to predict histological outcomes for assessing ligament healing (Ligament Maturity Index). Using the same ligament specimens from Specific Aim 2, hematoxylin and eosin (H&E) and alpha-smooth muscle actin antibody (SMA) stained sections for the ligaments were prepared and the slides were scored for a series of factors that are critical to ligament healing. Sub-scores based off the cell, collagen and vessel content were determined along with a total score (total LMI), representing the cumulative aspects of healing. We hypothesized that MR derived measures of T_2^* and volume would be significant predictors of the histological scoring of a healing ACL after 52 weeks of healing.

Median ligament T_2^* and volume significantly predicted semi-quantitative histological scores in healing bioenhanced repaired and naturally healing transected ligaments. In general, a shorter median T_2^* time and larger volume was associated with better histological outcome scores (Ligament Maturity Index, LMI) or indications of healing. MR ligament volume was not as strongly associated with histological outcomes as median T_2^* . This difference in prediction strength between median T_2^* and volume is likely due to the microscopic nature of the histological assessments. While few of the histological evaluation criteria account for gross ligament size, the majority of the criteria relate to elements of tissue organization, healing and remodeling at a microscopic level, which have been found to effect T_2^* times.[6,26] Our findings also align with a prior investigation relating low SI values to qualitative histological evidence of healing in ACL grafts for an ovine model.[25] The relationship between our MR variable T_2^* and histological scores tells a similar narrative to the qualitative findings of the previous ovine study. We found low T_2^* values were associated with greater cell density and collagen organization where the previous study found similar histological results for grafts with low SI.

Preceding histological analysis, mechanical testing was performed, during which the characteristic collagen crimp can flatten to a point of inelastic deformation.[11,21] To address this, a quasi-static protocol was used to test the ligaments (ramp speed 20mm/min) allowing the testing to be halted once a drop in load occurred to avoid complete disruption of the ligament and to minimize irreversible changes during the mechanical testing. During histological scoring, when choosing the five regions for histological analysis, the examiner selected regions that were free of synovium and not visibly deformed by biomechanical testing.[22] Furthermore, all ligaments in this study failed within the midsubstance and a defect was visible allowing histological assessment of the areas outside the area of local disruption. It is also reasonable to assume that the failure testing did not alter cellular or vascular content, so mechanical testing would have a limited impact on these histological sub-scores.

The use of T_2^* in Specific Aim 2 and Specific Aim 3 provides a critical step toward the improvement of a non-invasive method for predicting the structural properties or histological outcomes of a healing ligament *in vivo*. The use of T_2^* relaxation time which is an inherent tissue property, instead of signal intensity makes this approach less sensitive to image acquisition parameters, magnet manufacturer/hardware and is the first step to making this approach comparable across institutions.

While there are many advantages of a T_2^* based prediction model, current T_2^* methods require multiple-echo sequences at high-resolution. These three-dimensional, high-resolution images are essential for accurately characterizing small structures like the ACL. In a research setting, T_2^* maps generated from these high-resolution images are critical for identifying potential pathology and mapping regional variations as they relate to the biomechanical properties within a ligament. Currently, a voxel-wise multi-echo least squares fit is the gold standard to create T_2^* maps. Multi-echo least squares fit relaxometry maps have been proven to be accurate, minimally sensitive to noise and helpful to visualize regions of interest (ROI). Unfortunately, the post-processing associated with a least-squares fit is time-intensive (multiple hours) and thus difficult to implement clinically. However, the T_2^* fitting function could be modified to make it time appropriate for a clinical setting.

8.1.4 Specific Aim 4

Further refine and optimize the T_2^ relaxation time method from Specific Aim 2 & Specific Aim 3. Collect T_2^* data of the intact PCL using a 6 echo gold standard T_2^* method and two alternative methods for determining T_2^* and determine differences in fidelity and clinical feasibility between the methods.*

This aim was addressed in Chapter 5 of the thesis. The purpose of this study was to compare two alternative and computationally efficient methods of T_2^* determination (2MM and 6LS_{ROI}) to a time intensive gold standard (6LS). If these alternative methods could improve post-processing time without sacrificing the fidelity of T_2^* values, then the alternatives maybe valuable for eventual clinical translation.

We found the 2MM method overestimated T_2^* values while offering a regional T_2^* map for identifying spatial variations within a ligament. The 6LS_{ROI} method offered a comparable determination of ligament median T_2^* to the 6LS gold standard but could not offer a regional T_2^* map for identifying potential pathology. We found that theses alternative methods (2MM and 6LS_{ROI}) for determining median ligament T_2^* were by themselves limited. However, using them in tandem offered similar fidelity and visualtion

benefits in comparison to the traditional 6LS method, but in a fraction of the time (< 2 minutes for 2MM and 6LS_{ROI} combined; ~ 540 minutes 6LS gold standard).

With completion of this aim we have further refined our T_2^* methodology and developed an alternative method combining two alternative estimations of T_2^* . While the 6 echo gold standard (6LS) is still useful in a research setting, these two alternative approaches (2MM and 6LS_{ROI}), used in tandem, would take significantly less post-processing time than the gold standard, and could be combined to offer a tool for assessing ligament structural properties that would be viable in a clinical situation.

Looking at computational time and feasibility for T_2^* determination methods, and determining which methods are appropriate for a research setting or for potential clinical use is an important step for translating a prediction model between animal model research and the clinic. However, thus far it has been assumed that the MR parameters (volume, median SI and T_2^*) used to predict *ex vivo* structural properties or histological outcomes of the graft in an animal model would directly translate to a human clinical population. Unfortunately, *ex vivo* failure testing of the graft is not possible in a clinical population. Therefore, it may be possible to validate a method for non-invasively determining structural properties in an intact cadaveric human ACL model.

8.1.5 Specific Aim 5

Determine intact ligament T_2^ relaxation time and volume for a population of human cadaveric knees of varying age, and determine the ability of the MR variables to predict ACL structural properties for a degenerating ligament.*

This aim is discussed in Chapter 6. Our goal was to apply a T_2^* -based prediction model to a population of human cadaveric knees to determine if ligament degeneration could be predicted similar to ligament healing as presented in *Specific Aim 1 & Specific Aim 2*. 15 cadaveric knees were imaged *in situ* using a 6 echo determination of T_2^* . A range of ages was selected to capture different states of ligament degeneration, as the structural properties of the ligament have been shown to decrease with age.[27] Using the same structural properties protocol described in *Specific Aim 1 & Specific Aim 2*, the ligaments were then biomechanically assessed to determine structural properties. All specimens were identified as having a mid-substance failure. Similar to the healing ligament predictions (*Specific Aim 1 & Specific Aim 2*), we hypothesized that MRI derived volume and T_2^* outcome measures would correlate to *ex vivo* intact ACL structural properties of cadaveric specimens. Contrary to our hypothesis, it was found using volume

in conjunction with the median T_2^* value, the multiple linear regression model did not predict maximum failure load for this sample of intact human cadaveric ACLs; $R^2=0.23$, $p=0.200$. Similar insignificant results were found for yield load and linear stiffness.

Naturally restricted distributions of the intact ligament volume and T_2^* (demonstrated by the respective Z-scores) in an older cadaveric population may explain the insignificant results. Furthermore, previous studies that found a relationship between volume, T_2^* and ligament structural properties (*Specific Aim 2*) utilized a surgical animal model with different treatment groups of actively healing ligaments, resulting in a large range of evenly distributed volume and T_2^* data with little to no observed clustering. This stands in contrast to the current human cadaveric study, where small intact ligament volume was rare and the intact ligaments were not actively healing, leaving both the volume and T_2^* distributions naturally restricted. Naturally restricted distributions of MR variables observed in the intact ACL of a porcine model were also found to have a similar insignificant ability to predict structural properties when compared to a proven prediction in a healing ACL porcine model (Appendix C). Adding additional cadaveric specimens at younger and older ages may have captured a larger range of ligament degeneration and helped to better distribute the ligament volume and median T_2^* values and improve prediction strength.

While the MR variables did not significantly predict structural properties in a multiple regression, there was evidence that high volume and short T_2^* times were associated with higher structural properties. When the specimens were split into a low failure and high failure group (<700 N, $n=7$ and >700 N, $n=8$, respectively) and tested for differences in volume and median T_2^* using a one way ANOVA, we found the specimens in the high failure group had significantly lower T_2^* values in comparison to the low failure group (12.5 ± 1.4 vs 14.7 ± 2.4 ms, $p\text{-value}=0.048$). The high failure group tended to have larger volumes (1505 ± 594 vs 1182 ± 442 mm³, $p\text{-value}=0.261$) but the difference was not significant.

Although the same trends were observed with volume and T_2^* in the human cadaveric prediction compared to healing porcine ligaments (larger volume and shorter T_2^* times were associated with higher structural properties),[2] it is possible that there are inherent differences with the macro anatomy of the intact ligaments that led to the insignificant findings reported here. For instance the collagen fibers of the intact ACL twist in the intra-articular space and may cause volume-averaging and fiber orientation dependant artifacts that could confound the signal intensity or T_2^* values.[12,15] Furthermore, for this study we assumed that our T_2^* determination protocol would have similar sensitivity for detecting ligament degeneration as for assessing ligament healing. Unfortunately, the biological process involved in ligament healing is not the same as ligament degeneration. Scar formation, collagen remodeling and

vascular infiltration are critical for ligament healing and these processes are fundamentally different than ligament degeneration. With aging ligaments, cross linking, changes in collagen/water ratio and extracellular components such as glycosaminoglycan (gag) content are additional factors associated with degeneration and these factors may affect T_2^* times differently than the factors associated with healing.[1,6,19,20] Further research will be needed to clarify if these additional macro and microscopic factors may confound imaging results.

While the T_2^* values of human cadaveric ligaments were unable to predict degeneration in terms of structural properties, our imaging protocol may not be sensitive to the biological process of degeneration. However, we have shown in *Specific Aim 1* to *Specific Aim 3*, that the linear combination of graft volume and median SI or T_2^* can predict the structural properties or histological outcomes in a healing ligament animal model. Translating these methods for a healing ligament to a clinical population would be invaluable. Traditional outcome measures, such as the hop test, have been useful for many clinical studies as a standardized way to evaluate overall patient knee outcome following ACL treatment. However, these traditional evaluation techniques are knee specific measures of joint health and may lack the sensitivity to determine the biomechanical properties of the graft itself. Determining the relationship between a graft's MR parameters and a patient's knee specific traditional outcomes will offer a graft specific assessment to complement the existing clinical evaluation tools.

8.1.6 Specific Aim 6

Assess the structural properties prediction model from Specific Aim 1 as it relates to clinical, functional and patient-oriented outcome measures from an existing clinical ACL reconstruction trial.

The final aim was discussed in Chapter 7 of this thesis. The purpose of this study was to determine if the parameters of graft volume and signal intensity (SI), which have been used to predict the structural properties of the graft in an animal model (Specific Aim 1), correlate with commonly used traditional outcome measures such as, clinical, functional and patient-oriented outcome measures in patients 3- and 5-years after ACL reconstruction. We hypothesized that graft volume and signal intensity would correlate with measures of clinical, functional and patient-oriented outcome. Using a subset of participants enrolled in an ongoing ACL reconstruction clinical trial, AP knee laxity, 1-legged hop test, and KOOS scores were assessed at 3- and 5-year follow-up. 3-D high resolution MR images were also collected at each follow-up visit. Both the volume and median SI of the healing graft were determined and used as predictors

in a multiple regression linear model to predict the traditional outcome measures at each follow-up time separately. Graft volume combined with median SI in a multiple linear regression model predicted 1-legged hop test at both the 3-year and 5-year follow-up visits ($R^2=0.40$, $p=0.008$ and $R^2=0.62$, $p=0.003$, respectively). Similar significant results were found at 5-year follow-up for the KOOS sub-scores, though these variables were not significant at 3-year follow-up. The multiple linear regression model for AP knee laxity at 5-year follow-up approached significance.

In general, for traditional outcomes at 5-year follow-up, larger grafts with lower median SI values were associated with better knee performance and surgical outcome. For the functional outcome at 5-year follow-up, patients with higher hop% tended to have larger grafts with lower median SI. Similarly, for the KOOS sub-scores at 5-year follow-up, patients with larger graft volumes and lower SI had higher sub-scores.

These results show that MR parameters (volume and median SI) that relate to graft biomechanical performance or histological outcomes (*Specific Aim 1* to *Specific Aim 3*) are also predictive of overall patient knee health and ACL reconstruction surgical outcomes for patients at 3- and 5-year follow-up. Results from this study are a critical translational step that may enhance clinical evaluation of graft health. In a clinical setting a method relating the MR parameters of volume and median SI to traditional outcome measures could potentially aid researchers in determining the appropriate timing for athletes to return to sport.

8.2 Related studies and future directions

There are a number of related studies and future research directions that further support the body of work presented with the aims of this thesis. Developing the MR based ligament healing prediction model detailed in this thesis required the use of *ex vivo* and *in vivo* animal models, MR imaging pilot studies, statistical modeling analyses as well as, the use of patient clinical data. These unique opportunities led to a number of accessory studies that further bolstered the main research presented in this thesis. Related studies include testing the reliability of the MR metrics in *ex vivo* as well as *in vivo* imaging circumstances. An additional study tested the affects of restricted data distribution on prediction modeling. Another study addressed artifact minimization with the ACL reconstruction or repair procedure. The final studies discuss additional data that support the goal of the overall thesis but were outside the scope of the original prepared manuscripts. A summary of these supporting studies is presented below.

8.2.1 *Ex Vivo* MR Outcomes Reliability (Appendix A)

The objective of this study was to test the intra-examiner reliability of ligament or graft volume and signal intensity (SI) measures collected using MRI in both *ex vivo* human cadaveric and a porcine reconstruction models.

To test the reliability of the MR imaging and segmentation techniques used in this thesis, one human cadaveric knee with intact ACL and one reconstructed porcine knee were used. The same MR imaging protocol applied in Specific Aim 1 was used to image the human cadaveric knee seven times and the reconstructed porcine knee four times. The intact human ACL and reconstructed porcine graft were segmented from the MR image stacks and intra-articular volumes and median SI were determined for the ligaments. Within-subject coefficient of variation (WSCV) [24] was used to test the reproducibility of volume and SI measures across the repeated segmentations. The WSCV for volume in these repeated trials was 3.2% and 5.8% for the human knee and porcine knee, respectively. The WSCV for median SI in these repeated trials was 0.3% and 3.4% for the human and porcine knee, respectively. WSCV under 5.8% for both volume and signal intensity suggests that the measured volume and median SI values are reliable within the same examiner for both intact human cadaveric and porcine ACL reconstructed knees.[3]

8.2.2 *In Vivo* MR Outcomes Reliability (Appendix B)

The objective of this study was to test the impact of possible *in vivo* artifacts, such as movement and blood flow, on the feasibility and reproducibility of collecting ligament volume and T_2^* in a living porcine model.

To test the *in vivo* reliability of the MR imaging methods used in this thesis, one Yorkshire pig with intact knees was imaged. To handle space limitations associated with imaging an *in vivo* porcine model in a standard diameter clinical bore, a four channel flex coil was utilized. The six echo MR imaging protocol applied in Specific Aim 4 was used to image the intact porcine knee five times. Three-dimensional surface models of the intact ACL were created from the segmented images. T_2^* maps were then calculated using Matlab. From each of the five data sets, ligament histograms and summary statistics, including T_2^* median, were calculated. The whole ligament volume was then binned into four separate tissue sub-volumes (Vol_1 , Vol_2 , Vol_3 , Vol_4) with equal T_2^* intervals up to 50 ms (0-12.5; 12.6-25; 25.1-37.5;

37.6-50 ms, respectively) (Specific Aim 2). Tissue volume in terms of mm^3 was calculated for each sub-volume and the total ligament.

Within subject coefficient of variation (WSCV) [14] was used to test the inter-scan reliability ACL total volume, T_2^* summary statistics and sub-volumes defined by the range of T_2^* across the 5 data sets of the intact porcine ACL. WSCV was below 6.4% for total volume, sub-volumes, and T_2^* summary statistics for the five repeated scans of the intact porcine ACL. These results suggest that the measured volume and T_2^* values are reliable in an *in vivo* model.

8.2.3 Restricted Data Distribution on Prediction Strength (Appendix C)

As detailed in Specific Aim 5, the combination of volume and T_2^* could not predict the strength of an intact human cadaveric ACL. The limited distributions of volume and T_2^* data for this older human population could have affected the significance and strength of the prediction model for ligament structural properties. The objective of this study was to test the effect of a restricted volume or SI data distribution on the significance of a ligament structural properties prediction. To accomplish this, a proven prediction of healing ligament structural properties (Specific Aim 1) was compared to an unproven prediction of intact ligament structural properties in a porcine model. The intact ligaments were used to naturally restrict the volume and SI distributions and serve as a proxy for the distributions seen in the intact human ACL (Specific Aim 5).

As was reported in Specific Aim 1, the combination of ligament volume and median SI significantly ($p \leq 0.001$) and strongly ($R^2 = 0.73$) predicted maximum failure load in a healing porcine ligament. The same regression analysis combining volume and SI did not predict maximum failure load ($R^2 = 0.12$, $p = 0.111$) in an intact porcine ligament. This inability to predict failure load is likely due to the restricted volume and SI data distribution of the intact ligaments.[5,16] The operative ligaments had a volume range (1777.1 mm^3) more than three times as large as the intact ligaments (589.2 mm^3). Additionally, the range of SI data for the intact ligaments (1.7) was smaller than the operative ligaments (1.9). The large range and even distribution of both volume and SI data for the operative ligaments is due to the inclusion of two healing time points and four different ACL surgical procedures. Conversely, the group of intact ligaments was homogenous, which negatively affected the strength of the prediction in comparison to the surgical ligaments.[13,16] The data presented here indicates that limited availability of volume and SI data for the intact porcine ACL could have affected the significance and strength of the prediction model for intact ligament maximum load.

8.2.4 Artifact Minimization (Appendix D)

Artifact is a problem in MR imaging studies and is common following ACL reconstruction.[14,23] Artifacts, thought to be caused by metal particulate released from surgical instrumentation, can confound qualitative and quantitative outcome measures. The objective of this study was to recreate surgical conditions where artifact is created, in an effort to identify the cause of artifact and mitigate its effects. Two cadaveric porcine hind limbs were harvested at the hip joint. In a standard ACL reconstruction procedure, metallic instruments are used to create tunnels for placing the graft. Our goal was to identify artifact creation during tunnel creation, and to assess the effect of flushing the joint with saline on artifact appearance.

Artifact was identified during tunnel creation and was related to a combination of air bubbles and metal particulate. Artifact seen in the intact joints and the surrounding musculature indicates that artifact can result from collecting air bubbles. After tunnel creation, artifacts were also identified indicating that these artifacts were associated with metal hardware use. Flushing the joint only had a moderate effect on minimizing artifact appearance. To prevent artifact in an imaging study, considerations for minimizing both air and metal artifact creation are important. Minimizing air artifact would be a primary concern for *ex vivo in situ* imaging. Careful dissection could help stop the infiltration of air into the intra-articular space. Flushing the joint after tunnel creation could help prevent metal artifact. Identifying the cause of artifact in an ACL reconstruction procedure and mitigating its effects could greatly minimize data loss and significantly lower cost in MR imaging studies.

8.2.5 Volume and SI Predict Porcine Graft Laxity (Appendix E)

The purpose of this study was to determine if the same linear combination of volume and signal intensity used to predict porcine graft or ligament structural properties in Specific Aim 1 could also predict AP knee laxity at 15 and 52 weeks of healing. We hypothesized that the combination of volume and SI would also predict the graft or ligament AP laxity in a multiple regression model.

The same porcine reconstructed, repaired and transected knees used in the analysis of Specific Aim 1 were also tested for AP knee laxity before destructive structural properties testing. With the joint capsule intact the AP laxity values for the knees were measured using a custom fixture with the knee fixed at 30°, 60°, and 90° flexion (AP30, AP60, AP90, respectively).[5,6] These fixtures are meant to mimic a clinical AP knee laxity test, and to obtain laxity data similar to the clinical outcome gathered in Specific Aim

6. Anterior- and posterior-directed loads of ± 40 N were applied to the femur with respect to the tibia by an MTS 810 Materials Testing System (MTS, Prairie Eden, Minnesota), and the AP displacements were recorded.[6] Both volume and SI were included in a multiple linear regression model to predict AP knee laxity at 15 weeks and then at 52 weeks separately. The R-square and p-values for both the 15 and 52 weeks models were reported as indicators of the strength of the relationships. The combination of volume and SI were not able to predict AP60 at 15 weeks $R^2=0.13$ ($p=0.068$). Volume in conjunction with median SI, was able to significantly predict AP60 at 52 weeks of healing $R^2=0.56$ ($p\leq 0.001$).

In general, higher volume and/or lower SI measurements were associated with lower AP knee laxity of the reconstructed, repaired and transected ligaments. Prediction of graft or ligament AP laxity was stronger and significant at 52 weeks of healing than at 15 weeks of healing. This difference in prediction strength between the two time points indicates that there may be a lag time before graft or ligament strength is reflected in laxity measurements. This finding mirrors results found with Specific Aim 6 where patient graft volume and SI were not predictive of AP knee laxity gathered with a KT-1000 at 3-year follow-up but were predictive of AP laxity at 5-year follow-up. This signifies that time after surgery may play an important role in determining how graft health affects knee laxity.

8.2.6 Time Differences of MR Variables between 3- and 5-year follow-up (Appendix F)

In Specific Aim 6, at 5-year follow-up, the MR variables of volume and median SI were able to significantly predict hop% and KOOS sub-scores. The volume and median SI prediction for AP laxity approached significance at 5-year follow-up. At 3-year follow up, these same MR variables were unable to predict AP laxity and KOOS sub-scores, but were able to significantly predict hop%. Additionally, we saw an increase in prediction strength between the 3- and 5-year hop% data. This difference in prediction strength between the 3- and 5-year follow-up indicates that time after surgery may play an important role in determining how graft structural properties affects surgical outcome. The purpose of this study was to further evaluate the data from Specific Aim 6 by assessing the effect of time on patient graft MR and traditional outcome data. We tested the relationship between 3- and 5-year MR variables as well as the relationship between 3-year MR variables and future traditional outcomes at 5-year follow-up. As a baseline comparison, the relationship between the 3- and 5-year MR variables of intact ACLs from a set of age-matched control subjects [10] was also analyzed.

As hypothesized, 3-year graft volume predicted 5-year volume ($R^2=0.75$, $p<0.001$) and 3-year graft SI predicted 5-year SI ($R^2=0.46$, $p=0.005$). This indicates that the size and health of the graft at 3-year follow-up impacts the graft size and health two years later. Additionally, some patients had an increase in graft volume and SI, while some patients had a decrease in graft volume and SI between the follow-up times. This could indicate that patient grafts are still changing or remodeling up to the 5-year time point. We found that there were no detectable differences in MR volume or SI between 3- and 5-year follow-up for the control group. The lack of change in MR variables for the control group further indicates that observed changes in the grafts of ACL reconstruction patients is likely due to graft remodeling.

We also found that early graft MR variables (3-year Volume and SI) could significantly predict later patient outcomes at 5-year follow-up. This indicates that the health of the graft at earlier time points impacts later traditional outcome. This maybe particularly important if this relationship applies to even earlier time points. For instance if graft health in terms of MR variables at 1-year follow-up can predict later traditional outcomes at 5-years, this relationship could help influence clinical intervention for patients with poor MR outcomes at early time points. In conclusion, the relationship of 3-year MR variables to 5-year MR variables and traditional patient outcomes may indicate that the graft continues to remodel, and signifies that time after surgery plays an important role in determining how graft structural properties affects traditional outcomes.

8.3 Future Directions

8.3.1 Longitudinal Validation of MR Prediction Methods

The studies presented in this thesis were included as part of a grant application that has been awarded (NIH 1R01-AR065462; Fleming/Murray). Future research funded through this grant will focus on further validation of the MR-based method to predict the biomechanical performance of a healing ligament discussed in this thesis. This study will use two aims to address how the MR-based prediction method will 1) change with early phases of ligament healing and 2) how early stages of healing affect later biomechanical outcomes within a ligament. To address the first aim a cross sectional study will be used to determine the relationships between MR parameters and biomechanics at multiple early stages of healing to further optimize the prediction model. The second aim will use a longitudinal study to document changes in ligament biomechanics using the MR-based prediction approach to independently

validate the model's ability to discriminate between treatments and to determine if scans obtained in the early stages of healing will predict the later biomechanical outcomes. For this study, we will focus on the 6 to 24 week healing window as this period encompasses early healing phases and the range of greatest improvement in the ligament biomechanical properties [4,7,17,18]. At the completion of this study, we will have optimized our MR-based prediction model to document healing following the surgical treatment of an ACL injury, will be positioned to adapt this model to other healing ligaments and tendons, and will be poised to perform pre-clinical and clinical trials comparing bio-enhanced ACL repair with the current gold standard of treatment.

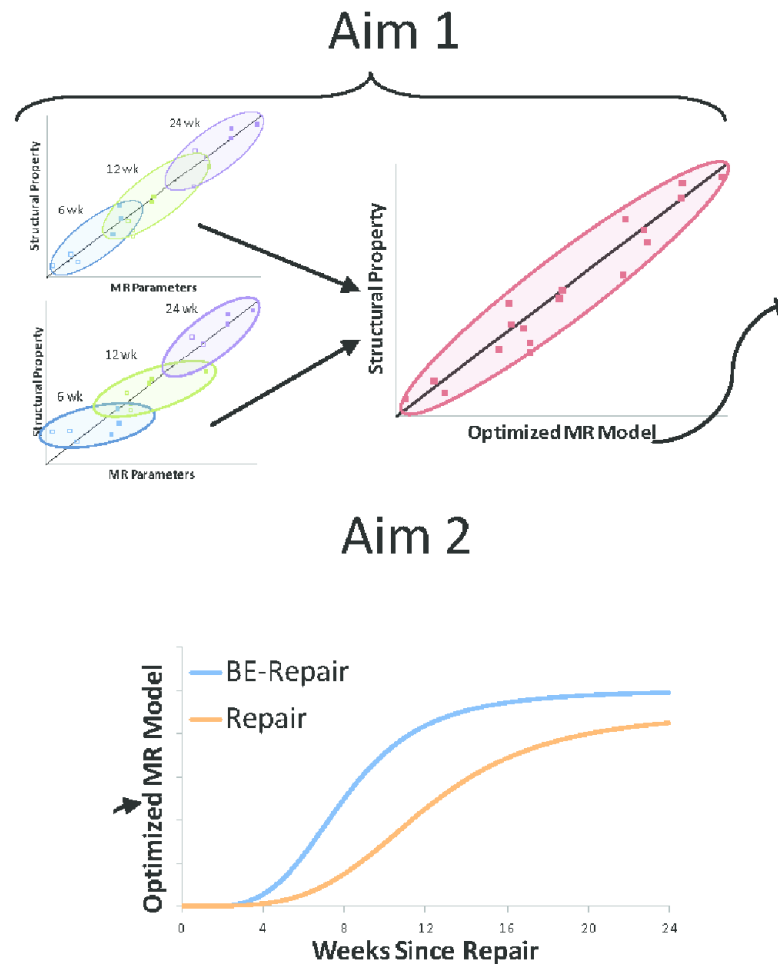


Figure 8.1: In Aim 1 the model will be optimized to include the temporal changes and in Aim 2 it will be applied to longitudinally document changes in healing of two repair strategies to determine if measurements in the early stages of healing are predictive of those in the later stages.

8.3.2 MR to Predict Collagen Content in a Degenerating Ligament

The seed grant that funded Specific Aim 5 will also fund future research looking to relate intact ACL volume and T_2^* to histological outcomes in a group of osteoarthritis (OA) patients. Patients with advanced OA are often treated with a total knee arthroplasty (TKA), where the articular surfaces of the knee are removed and replaced with prosthesis. This study will take advantage of the unique opportunity that a TKA patient population provides to validate our MR approach in humans since the knee articular surfaces are discarded along with the cruciate ligaments at the time of surgery. This circumstance will allow *in vivo* imaging in patients to determine the MR variables while permitting *ex vivo* histological analysis. The combination of *in vivo* imaging and *ex vivo* analysis may help alleviate some challenges encountered with intact ACL imaging previously done in a human cadaveric model (Specific Aim 5). The initial stages of this study have been completed. The knees of these patients have been imaged prior to their replacement surgery and the MR images have been processed to determine the volume and T_2^* values of the ligaments *in vivo*. The ligaments were then harvested at the time of surgery by our collaborator Dr. Lee Rubin and have been prepped for subsequent histological analysis. This study seeks to further clarify the research goals of Specific Aim 3 & Specific Aim 5 by determining how MR metrics of the intact but degenerating ACL in OA patients affect histological outcomes.

8.4 Summary

The goal of this thesis was to use the quantifiable MR parameters of volume, signal intensity and T_2^* relaxation time to predict the biomechanical and histological outcomes of an ACL graft or ACL repair as a surrogate outcome measure for healing. The main focus was on developing imaging methods for determining these MR parameters and evaluating how these parameters could be used to assess ligament or graft healing, in both animal models and in clinical trials. Additionally, a complimentary study determined that these same imaging parameters were not significantly associated with biomechanical performance of degenerating ACLs in a human cadaveric population clustered around 50 years of age. Nonetheless, with the completion of the aims of this study, we have developed an MR imaging technique tailored to the healing ligament or graft and assessed its ability to be used as a quantitative outcome measure of ACL integrity.

This non-invasive MR method for predicting the structural properties and histological outcomes of the graft or ligament will allow researchers to document functional healing longitudinally within a specimen in pre-clinical animal studies. Furthermore, these findings may have implications as a surrogate outcome measure in clinical studies for documenting temporal changes within-patients and as a more quantitative method for guiding rehabilitation and determining when a patient is ready to return to high level activity.

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Appendix A

***Ex Vivo* Intra-Examiner Reliability of the MR Imaging and Segmentation Technique Used to Obtain Volume and Signal Intensity Parameters**

Alison M. Biercevicz

A.1 Objective

The objective of this study was to test the intra-examiner reliability of ligament or graft volume and signal intensity (SI) measures collected using MRI in both ex vivo human cadaveric and a porcine reconstruction models.

A.2 Methods

To test the reliability of the MR imaging and segmentation techniques used in this thesis, one human cadaveric knee (female age 54) with intact ACL and one patellar tendon allograft reconstructed porcine knee (52 weeks of healing) were used. A surface knee coil on a 3T MR scanner (Siemens Trio, Erlangen, Germany) was used to image the joints. A T_2^* weighted 3D-CISS sequence (Constructive Interference in the Steady State; TR/TE/FA, 12.9/6.5/ 35°; FOV, 160 mm; matrix 512X512, slice length/gap, 0.8mm/0; avg 1) was selected. This sequence produces high contrast between the soft tissues and joint fluid, [3,4] which optimizes the boundaries of the ligament or graft for manual segmentation from the image stack. The intact human cadaver knee was imaged 7 times while the reconstructed porcine knee was imaged 4 times. Between scans the knees were removed from the scanner, flexed and extended 10 times and placed back in the scanner to create unique coordinates and slice locations for each repeated scan.

Using commercially available software (Mimics 13.1, Materialise, Ann Arbor, Michigan), the intact human ACL and reconstructed porcine graft were segmented from the MR image stacks (Figure A.1). The same examiner with was used for all segmentations and all the scans were batch processed within 2 weeks time to limit variability. Three-dimensional surface models and SI volumes were created from the segmented images on a pixel by pixel basis. The median SI from the ligament were normalized to the signal intensity of femoral cortical bone to account for inter-scan variability.[2] Intra-articular volumes and median SI were determined for the ligaments.

A.3 Statistics

The within-subject coefficient of variation (WSCV) was used to test the reproducibility of the measurements [5] and was calculated for the volume and median SI measures across the seven segmentations of

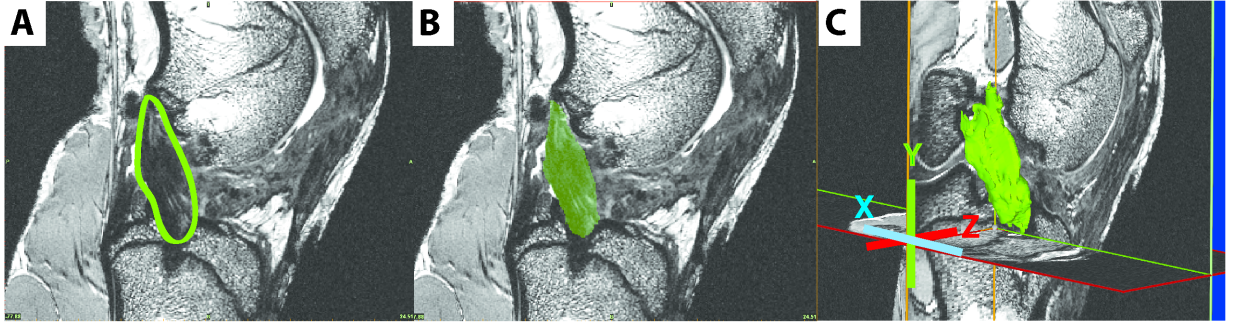


Figure A.1: 3D segmentation process illustrated on one slice of the image stack. A) 2D graft location; B) Segmented graft; C) 3D model of the graft. Figure from Biercevicz, A. M., Miranda, D. L., Machan, J. T., Murray, M. M., and Fleming, B. C., In Situ, noninvasive, T2*-weighted MRI-derived parameters predict ex vivo structural properties of an anterior cruciate ligament reconstruction or bioenhanced primary repair in a porcine model, *The American Journal of Sports Medicine* 41 (2013) 560–566.

the ACL intact human knee and the four segmentations of the reconstructed porcine knee.

A.4 Results and Discussion

The mean volume and median SI values for the 7 repeated ACL intact human knee scans were $1288.8 \pm 40.9 \text{ mm}^3$ and 5.39 ± 0.3 , respectively. The WSCV for these repeated trials were 3.2% and 5.6%, respectively (Table A.1).

Image Set #	Total Vol (mm^3)	Median SI
1	1330.1	5.73
2	1331.1	5.53
3	1309.6	5.60
4	1214.9	5.00
5	1315.1	5.00
6	1252.8	5.60
7	1289.2	5.25
mean	1288.8	5.39
stdev	40.9	0.3
COV%	3.2	5.6

Table A.1: For the seven human cadaveric knee image sets: Mean, standard deviation and coefficient of variation (COV) for the ligament total volume and median SI.

The mean volume and median SI values for the 4 repeated porcine reconstructed ligament scans were $1729.5 \pm 100.0 \text{ mm}^3$ and 1.30 ± 0.04 , respectively. The WSCV for these repeated trials were 5.8 and

3.6%, respectively (Table A.2).

In a meniscal volume soft tissue reproducibility study, utilizing T_2^* weighted images collected with the CISS sequence, a WSCV around 4% was observed [1]. WSCV under 5.8% for both volume and signal intensity measures from the seven repeated scans of the intact human cadaveric knee and the four repeated scans of the porcine ACL reconstructed knee suggest that the measured volume and median SI values are reliable with-in the same examiner.

Image Set #	Total Vol (mm ³)	Median SI
1	1668.3	1.31
2	1645.5	1.39
3	1736.4	1.29
4	1867.8	1.29
mean	1729.5	1.30
stdev	100.0	0.04
COV%	5.8	3.6

Table A.2: For the four porcine reconstructed knee image sets: Mean, standard deviation and coefficient of variation (COV) for the ligament total volume and median SI.

A.5 Conflicts of interest

None.

A.6 Acknowledgements

A.7 References

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Appendix B

Evaluation of the *In Vivo* Reliability of Anterior Cruciate Ligament (ACL) MR Volume and T_2^* Values in a Porcine Model

Alison M. Biercevicz, Braden C. Fleming

B.1 Objective

The objective of this study was to test the impact of possible *in vivo* artifacts, such as movement and blood flow, on the feasibility and reliability of collecting ligament volume and T_2^* in a living porcine model.

B.2 Methods

To test the reliability of the proposed MR imaging, one adolescent Yorkshire pig (male, age 3 months, 53kg) with intact knees was used. To handle space limitations associated with imaging an *in vivo* porcine model in a standard diameter clinical bore, a four channel flex coil was utilized on a 3T MR scanner (Siemens Trio, Erlangen, Germany) to image the animal's right knee (Figure B.1).



Figure B.1: Imaging set up for space limitations associated with a porcine model. The animal was positioned on its side with the caudal end of the animal inside the bore. A four channel flex coil was used to obtain high resolution images of the field of view while limiting scan time.

A high resolution 3D T_1 -weighted FLASH sequence, utilizing 6-echo times, (TR/TE/FA, 33/4.3, 7.3, 10.2, 13.1, 16.0 & 18.9/ 17°; FOV, 180 mm; matrix 512X512, slice length/gap, 0.8mm/0; avg 1; bandwidth 407) was acquired 5 times. Imaging time for each scan was approximately 20 minutes. In between image set acquisitions the flex coil was removed and the animal's knee was flexed and extended 10 times. Then the knee and flex coil were repositioned in the scanner and imaging was repeated.

Using commercially available software (Mimics 13.1, Materialise, Ann Arbor, Michigan), the entire volume of the intact porcine ACL was segmented from each image set. Three-dimensional surface models were created from the segmented images. T_2^* maps were then calculated using custom Matlab code, the 6 echos from each data set were used for a voxel-wise nonlinear least-squares fit of voxel intensity versus echo time for T_2^* estimation.[2] The ACL voxels were extracted from the maps using the models created from the segmented images (Figure B.2).

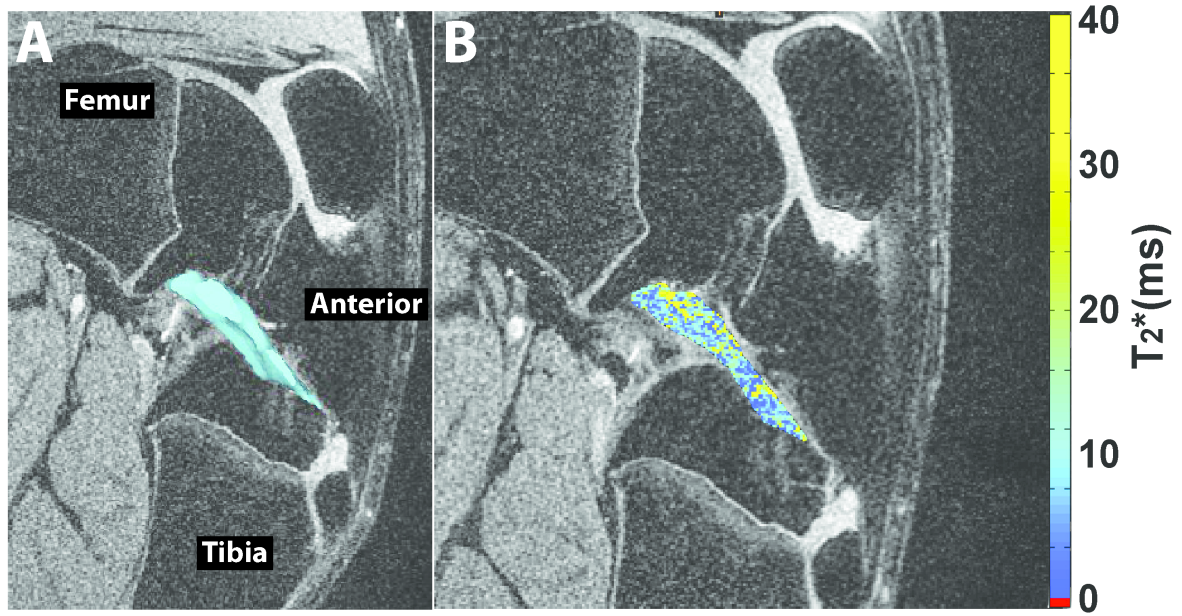


Figure B.2: A. Three-dimensional surface model of the segmented ACL overlaid on the original DICOM (sagittal view of the knee). B. The same sagittal view of the knee with a T_2^* ligament specific map overlay. The range of T_2^* values can be seen on the right side of the image.

From these ligament specific maps, histograms of the voxel-wise T_2^* were plotted. From each of the five data sets ligament histograms, summary statistics (mean, median, 1st quartile (Q1) and 3rd quartile (Q3)) were calculated for each method. Additionally, the whole ligament volume was then binned into four separate tissue sub-volumes (Vol_1 , Vol_2 , Vol_3 , Vol_4) with equal T_2^* intervals up to 50 ms (0-12.5; 12.6-25; 25.1-37.5; 37.6-50 ms, respectively) (Figure B.2). Tissue volume in terms of mm^3 (note: for this MR protocol 10.1 voxels equaled one mm^3) was calculated for each sub-volume.

B.3 Statistics

The within subject coefficient of variation (WSCV) was used to test the inter-scan reliability of the measurements [3] and was calculated for the ACL total volume, T_2^* summary statistics and sub-volumes defined by range of T_2^* across the 5 data sets of the intact porcine ACL.

B.4 Results and Discussion

The mean sub-volume (Vol_1 , Vol_2 , Vol_3 , Vol_4) and total volume values for the 5 repeated ACL intact human knee scans were $476 \pm 17.6 \text{ mm}^3$, $274 \pm 14.8 \text{ mm}^3$, $78 \pm 7.5 \text{ mm}^3$, $43 \pm 6.1 \text{ mm}^3$ and $871 \pm 24.0 \text{ mm}^3$, respectively. The WSCV for these repeated trials were 3.7%, 5.4%, 9.6%, 14.2% and 2.8%, respectively (Table B.1).

Image Set #	$Vol_1(\text{mm}^3)$	$Vol_2(\text{mm}^3)$	$Vol_3(\text{mm}^3)$	$Vol_4(\text{mm}^3)$	Tot Vol (mm^3)
1	474.81	239.48	68.72	42.02	825.03
2	461.66	277.84	86.02	48.25	873.78
3	491.42	292.68	76.43	34.80	895.33
4	490.92	258.17	81.87	47.26	878.23
5	459.88	267.46	68.62	42.32	838.28
mean	475.97	274.04	78.24	43.16	871.40
stdev	17.57	14.80	7.52	6.14	23.96
WSCV%	3.69	5.40	9.61	14.24	2.75

Table B.1: For the five image sets: Mean, standard deviation and within subject coefficient of variation (WSCV) for the ligament total volume and sub-volumes defined by range of T_2^* .

The T_2^* summary statistics (Mean, Median, Q1, Q3) for the 5 repeated porcine scans were 14.0 ± 0.4 ms, 11.4 ± 0.4 ms, 5.8 ± 0.4 ms and 18.4 ± 0.4 ms, respectively. The WSCV for these repeated trials were 2.7%, 3.3%, 6.4% and 2.3%, respectively (Table B.2).

In a meniscal volume soft tissue reliability study, utilizing T_2^* weighted images collected with the CISS sequence, a WSCV around 4% was observed.[1] WSCV under 6.42% for total volume, sub-volumes, and T_2^* summary statistics from the five repeated scans of the intact porcine ACL knee suggest that the measured volume and median grayscale values are reproducible.

Image Set #	T ₂ * Mean (ms)	T ₂ * Median (ms)	T ₂ * Q1 (ms)	T ₂ * Q3 (ms)
1	13.48	10.59	5.36	17.86
2	14.50	11.87	6.37	18.91
3	13.62	11.26	5.82	18.08
4	13.95	10.96	5.50	18.52
5	13.83	11.40	5.68	17.97
mean	13.97	11.38	5.84	18.37
stdev	0.37	0.38	0.38	0.43
WSCV%	2.67	3.33	6.42	2.34

Table B.2: For the five image sets: Mean, standard deviation and within subject coefficient of variation (WSCV) for the ligament T₂* summary statistics (mean, median, 1st and third quartiles (Q1 & Q3)).

B.5 Conflicts of interest

None.

B.6 Acknowledgements

Brown Magnetic Resonance Facility (MRF) and Brown Animal Care Facility (ACF) for their assistance with the *in vivo* imaging

B.7 References

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Appendix C

Effect of a Restricted Data Distribution on Prediction Strength

Alison M. Biercevicz, Braden C. Fleming

C.1 Introduction

The linear combination of volume and SI or T_2^* has been found to significantly predict healing ligament strength in a porcine ACL reconstruction and repair model.[2,3] Using the same prediction model approach it was found that the combination of volume and T_2^* could not predict the strength of an intact human cadaveric ACL (Chapter 6). However, the distributions of volume and T_2^* for these intact human ligaments were limited in range and were clustered around the median value for the data set. This limited availability of volume and T_2^* data for this human population could have affected the significance and strength of the prediction model for intact ligament structural properties.

C.2 Objective

The objective of this study is to test the effect of a limited volume or SI data distribution on the significance of a ligament structural properties prediction (SI is correlated to T_2^* and similarly represents ligament integrity). To accomplish this, a prediction of healing ligament structural properties, that has been proven in an operative porcine model (Specific Aim 1), will be compared to a prediction of intact ligament structural properties. Intact ligaments will be used to naturally restrict the volume and SI distributions, similar to the human ACL study. These naturally restricted distributions will serve as a proxy to the distributions seen in the intact human ACL (Specific Aim 5).

C.3 Methods

As part of a study investigating the efficacy of ACL reconstruction, sixty-eight adolescent Yucatan minipigs (approximately 15 weeks of age) underwent an ACL transection, reconstruction or repair procedure as previously described.[6] After 15 weeks of healing, 42 of the animals were euthanized and the operative as well as the control intact legs were harvested. Following 52 weeks of healing, the remaining 26 animals were euthanized and only the operative legs were harvested for MR imaging. The inclusion of two healing time points and different ACL surgical procedures helped create a large range of volume and SI data for the operative healing ligaments. MR imaging was performed immediately after harvest on both the operative and intact knees before the joints were frozen and stored for mechanical testing. The healing ligament volume and SI data from the healing ligaments was analyzed and published as a proven prediction model for ligament strength (Specific Aim 1).

C.3.1 Imaging

A surface knee coil on a 3T MR scanner (Siemens TIM Trio, Erlangen, Germany) was used to image the joints. A T_2^* weighted 3D-CISS sequence (Constructive Interference in the Steady State; TR/TE/FA, 12.9/6.5/ 35°; FOV, 160 mm; matrix 512X512, slice length/gap, 0.8mm/0; avg 1) was selected. Using commercially available software (Mimics 13.1, Materialise, Ann Arbor, Michigan), both the operative and intact ligaments were manually segmented from the MR image stacks. Three-dimensional surface models and SI grayscale volumes were created from the segmented images. Intra-articular volumes and SI (in terms of median ligament SI) were determined for the operative and intact ligaments. The SI values from the operative and intact ligaments were normalized to the SI value of femoral cortical bone to account for inter-scan variability.[4,10]

C.3.2 Structural Properties

The same tensile testing protocol used in 1.3.1, 1.3.2, and 1.3.5 was used here to determine the structural properties of both the operative and intact porcine ACLs.[1–3,6,9] The specimens were thawed to room temperature. The femur was transected just distal to the hip while the tibia was transected just proximal to the ankle to preserve the length of the long bones. The soft tissues were dissected from the tibia and femur while leaving the joint capsule intact. The proximal end of the femur and the distal end of the tibia were potted in 6 inch and 4 inch lengths of 1½" PVC pipe, respectively, using a urethane resin (Smooth-On, Easton, Pennsylvania). All residual soft tissue and joint structures were then removed from the joint leaving only the femur-ligament-tibia complex intact. Using a servohydraulic material testing system (MTS 810; Prairie Eden, MN), the tensile loads were applied at 20mm/min to failure as previously reported.[9] Initially, the joint was placed at 30 degrees of flexion so that the mechanical axis of the ligament was collinear with the direction of pull of the tensile testing actuator. Starting with a tibiofemoral compressive force of 5 N, the entire load-displacement curve was recorded until a precipitous drop in load occurred. Yield load, maximum load, and linear stiffness values of the ligaments were calculated as previously described.[9] The specimens were thawed to room temperature. The proximal end of the femur and the distal end of the tibia were potted in PVC pipe using a urethane resin. The joint was carefully dissected, leaving only the femur-ligament-tibia complex intact. Using a servohydraulic material testing system (MTS 810; Prairie Eden, MN), the tensile loads were applied at 20 mm/min to failure as previously reported.[9] Starting with a tibiofemoral compressive pre-load of 5 N, the entire tensile load-displacement curve was recorded until a precipitous drop in load occurred. The linear stiffness,

maximum load and yield load values of the operative and intact ligaments were calculated from the load-displacement data.[9]

C.3.3 Statistics

C.3.3.1 Summary Statistics

Summary statistics (maximum, minimum, median, skewness) for the volume and SI data were calculated (Table C.1) for the operative and intact ligaments separately. These summary statistics were used to assess the shape and distribution of the data sets for both the operative and intact ligaments.

C.3.3.2 Prediction models

For both the operative and the intact ligaments, linear regression models were used to separately test the relationships between: a) MR volume and structural properties and, b) SI and structural properties. Subsequently, both volume and SI were included in a multiple linear regression model to predict the structural properties (a fit plane) (SigmaPlot 12.0; Systat Software Inc., San Jose, CA) of the operative and intact ligaments separately. The resulting additive models included a volume term (VOL), representing the total ligament volume and ligament median SI (Table C.2). The R-square and p-values were reported as indicators of the relationship strength and goodness of fit for both the operative and intact ligaments.

C.4 Results

C.4.1 Summary Statistics

For the operative healing ligaments the maximum, minimum, and median volume were 1883.6, 106.5, and 944.0 mm³, respectively. The maximum, minimum, and median SI for the operative ACL data set was 1.90, -0.002, and 0.94, respectively. The maximum, minimum, and median maximum load for the operative ACL data set was 1806.4, 32.0, and 649.3 N, respectively (Table C.1).

For the intact ligaments the maximum, minimum, and median volume was 1216.4, 618.2, and 866.5 mm³, respectively. The maximum, minimum, and median SI for the intact ligaments data set was 2.0,

0.69, and 1.5, respectively. The maximum, minimum, and median maximum load for the intact ligaments data set was 2114.9, 949.5, and 1531.8 N, respectively (Table C.1).

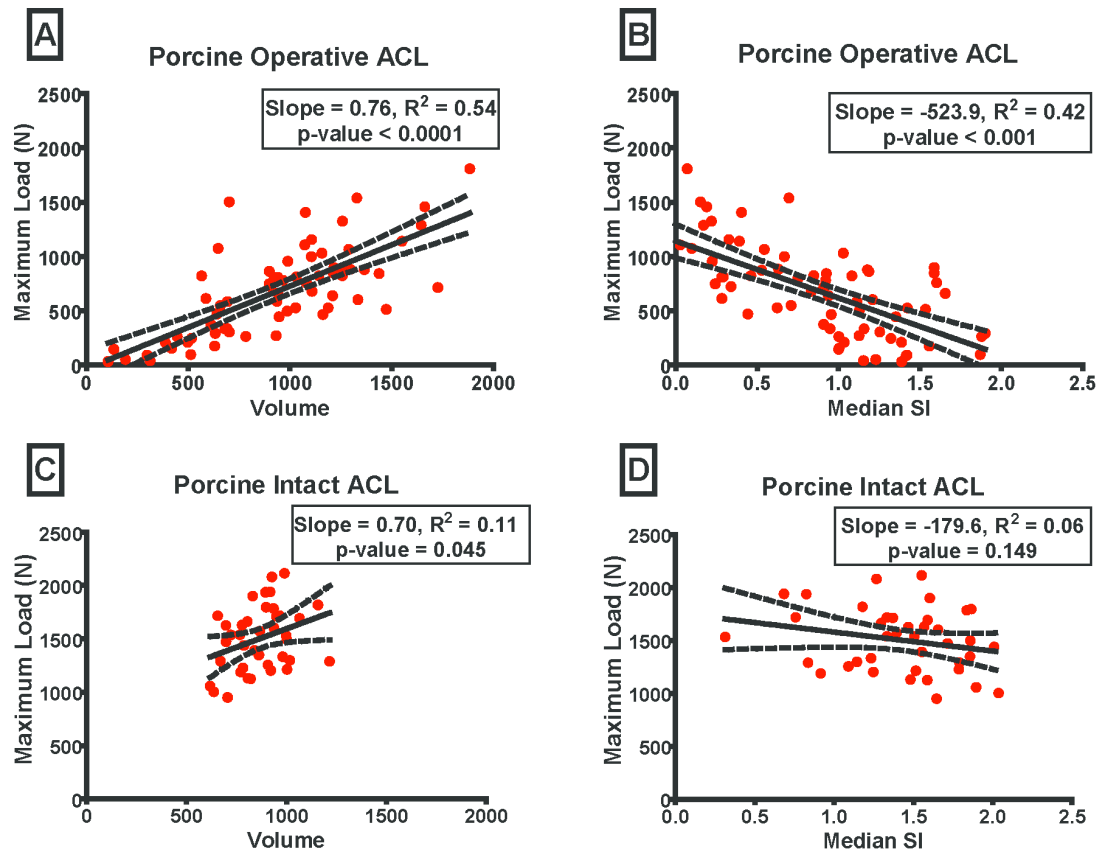


Figure C.1: The maximum load for the operative and intact ligaments as a function of ligament volume and the median SI in the linear regression models. Dashed lines represent 95% confidence interval.

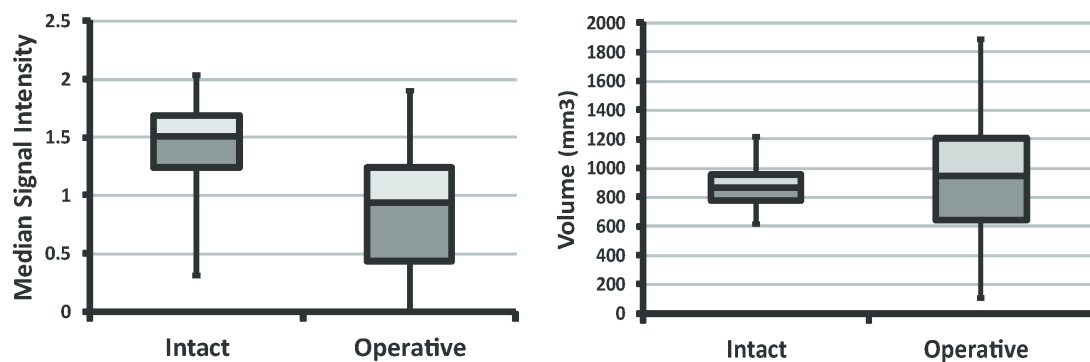


Figure C.2: Box and whisker plots (25-75% confidence intervals and maximum/minimum values) for median SI and Volume for both intact porcine ligaments and surgical operative ligaments.

C.4.2 Prediction models

The volume of operative ligaments significantly predicted maximum failure load ($R^2=0.54$, $p\leq 0.001$). The median SI value significantly predicted maximum failure load ($R^2=0.42$, $p\leq 0.001$) (Table C.2). Using volume in conjunction with the median SI value, the multiple linear regression model showed an increased ability to significantly predict maximum failure load for the operative ligaments; ($R^2=0.73$, $p\leq 0.001$) (Table C.2).

The volume of intact ligaments did significantly but not strongly predict maximum failure load ($R^2=0.11$, $p=0.045$). The median SI value did not predict maximum failure load ($R^2=0.06$, $p=0.149$) (Table 2). Using volume in conjunction with the median SI value, the multiple linear regression model did not significantly predict maximum failure load for the intact ACL ($R^2=0.12$, $p=0.111$) (Table C.2). It should be noted that, during mechanical testing, all ligament specimens failed mid-substance.

Model	Independent Variable	Range	Max	Min	Median	Skewness
Porcine OP ACL	VOL	1777.1	1883.6	106.5	944.1	0.0149
	Median SI	1.90	1.90	0.00	0.94	0.00343
	Max Load (N)	1774.4	1806.4	32.0	669.1	0.466
Porcine Intact ACL	VOL	598.2	1216.4	618.2	866.5	0.263
	Median SI	1.72	2.04	0.31	1.51	-0.788
	Max Load (N)	1165.4	2114.9	949.5	1531.8	0.132

Table C.1: Summary statistics for the operative (OP) and intact ligament volume, SI, and maximum load data sets.

Model	Dependant Variable	Independent Variable	R^2	Regression p-value	Slope	Intercept
Porcine OP ACL	Max Load (N)	VOL	0.54	<0.001	0.76 ± 0.09	-32.2 ± 86.6
	Max Load (N)	Median SI	0.42	<0.001	-523.9 ± 77.7	1142 ± 77.6
	Max Load (N)	VOL	0.73	<0.001	0.61 ± 0.07	440.1 ± 96.7
		Median SI			-370.1 ± 54.8	
Porcine Intact ACL	Max Load (N)	VOL	0.11	0.045	0.69 ± 0.33	903.0 ± 292.2
	Max Load (N)	Median SI	0.06	0.149	-179.6 ± 121.8	1759.0 ± 180.0
	Max Load (N)	VOL	0.12	0.111	0.58 ± 0.37	1121.7 ± 445.7
		Median SI			-87.2 ± 133.4	

Table C.2: Summary of the maximum load prediction equations for both porcine operative (OP) and intact ligaments as a function of volume (VOL) and median SI.

C.5 Discussion

As was previously reported (Specific Aim 1), the combination of operative ligament volume and median SI significantly ($p \leq 0.001$) and strongly ($R^2 = 0.73$) predicted maximum failure load. The same regression analysis combining intact ligament volume and SI did not predict maximum failure load ($R^2 = 0.12$, $p = 0.111$). This inability to predict failure load is likely due to the limited range of volume and SI data seen with the intact ligaments.[5,8] The operative ligaments had a volume range (1777.1 mm^3) more than three times as large as the intact ligaments (589.2 mm^3) (Figure C.1, Table C.1). Additionally, the range of SI data for the intact ligaments was smaller than the operative ligaments (Table C.1) and the distribution of SI values in the intact ligament data set was less evenly distributed. This can be seen by the negative skew (-0.788 , clustering of data towards higher SI values) of the intact ligament SI, where the operative ligaments displayed little to no skew (0.003 , even distribution across the range) (Table C.1, Figure C.1). The large range and even distribution of both volume and SI data for the operative ligaments is due to the inclusion of two healing time points and different ACL surgical procedures. Conversely, the limited variability of volume and SI data seen with the intact ligaments was due to the animals coming from the same healthy porcine population of similar age. As a result, both the independent variables (volume and SI) in the intact ligaments had a restricted range or were homogenous, which negatively affected the strength of the prediction in comparison to the surgical ligaments that had a naturally heterogeneous composition for the independent variables.[7,8]

While the predictions for the intact ligaments were not strong and significant, the same trend of high volume and low SI measures being associated with high maximum load was identified. However, in the predication equations of the intact ligaments the slope of median SI was significantly lower than the operative ligaments (Table C.2). Furthermore, the y-intercept of the intact ligament predictions was offset to higher values (Table C.2), in comparison to the operative ligaments. This could indicate that while the same trends were identified with the intact ligament predictions, there could be an inherent difference with the biology or anatomy of the intact ligaments that creates these differences. These prediction differences (slope and intercept) could also be an artifact of the limited volume and SI data seen with the intact ligaments.

There are some limitations to this study. Intact limbs were only included from the 15 week operative animals ($n = 42$). MRI was not gathered of the intact limbs from the 52 week time point of the original study, so analysis of the intact limbs from this time point was not possible. The inclusion of animals that were 37 weeks older with presumably larger ligaments would have had a limited effect on the predictability

of the intact ligaments. The volume data for the intact specimen prediction was sparse at the low end of the range and more intact specimens with larger volume would have a limited affect on the prediction. Second, the SI variable used to represent tissue integrity can vary depending on MR imaging parameters. We normalized the grayscale values to that of cortical bone within each image to minimize this concern. Despite these limitations, the data presented here indicates that limited availability of volume and SI data for the intact porcine ACL could have affected the significance and strength of the prediction model for intact ligament maximum load.

C.6 Conflicts of interest

None.

C.7 Acknowledgements

Jason Machan and Gary Badger for their guidance on statistics

C.8 References

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Appendix D

The Creation and Mitigation of Artifact with the Anterior Cruciate Ligament (ACL) Reconstruction Procedure in an *Ex Vivo* Porcine Model

Andrew C. Rohan, Alison M. Biercevicz, Braden C. Fleming

D.1 Introduction

Artifact is a problem in MR imaging studies and is common following ACL reconstruction.[2,3] Artifacts, thought to be caused by metal particulate released from surgical instrumentation, can confound qualitative and quantitative outcome measures. Specifically in efficacy studies of new ACL treatment procedures, these artifacts have resulted in lost MR data points and wasted resources for both animal model[1] and human clinical research.[2] Decreasing the incidence of artifact and its effect on MR outcomes would minimize data loss, decreasing the number of animals or patients required per study group and significantly lowering cost. The objective of this study was to recreate surgical conditions where artifact is created, in an effort to identify the cause of artifact and mitigate its effects.

D.2 Methods

Two cadaveric porcine hind limbs harvested at the hip joint were used (~25 kg animal). Each knee was imaged intact to create baseline images for comparison. In a standard ACL reconstruction procedure, two basic steps are used to create the tunnels for placing the graft. These two steps involve drilling a flexible passing pin from the cortical bone into the intra-articular space and then drilling over the pin with a cannulated drill bit to create the tunnel into the intra-articular space. Our goal was to identify artifact creation during tunnel creation, and to assess the effect of flushing the joint with saline on artifact appearance.

D.2.1 Passing Pin

Care was taken to keep the joint capsule intact to avoid infiltration by air bubbles, which can induce MR artifact. The first step was to drill a passing pin (Smith and Nephew Endoscopy, Andover, MA) into the intra-articular space. This was done four times through the lateral tibia, medial tibia, lateral femur and medial femur (Figure D.1). A new passing pin was used for each knee, but the same passing pin was used for each of the 4 tunnels. Imaging was then repeated to identify if artifact was present. Artifact was identified with the first joint and no artifact was identified in the second.

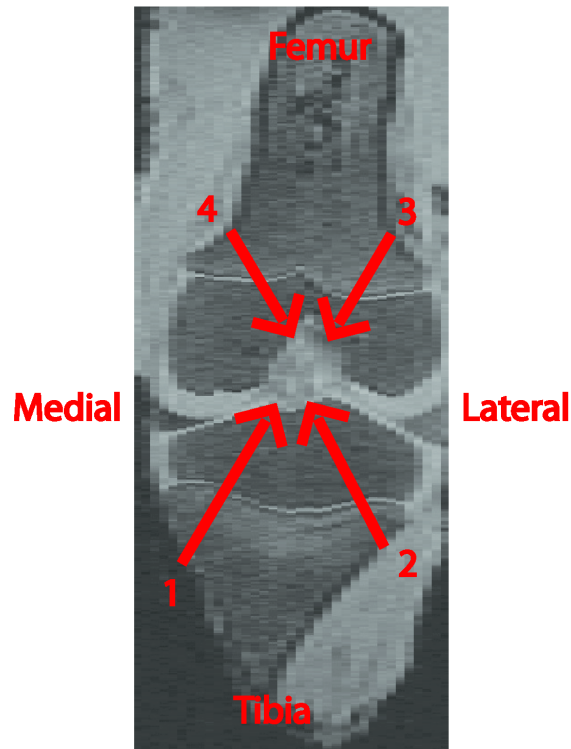


Figure D.1: Schematic showing path of passing pins and or cannulated drill bits into the intra-articular space.

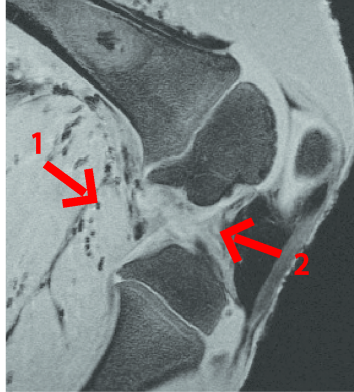
D.2.2 Cannulated drill bit

With the second knee, a cannulated drill bit (Smith and Nephew Endoscopy, Andover, MA) was used to drill over the passing pin tunnels; this was done for all 4 tunnels. This knee was then re-imaged and artifact was identified. The cannulated drill bit was only used on the second joint.

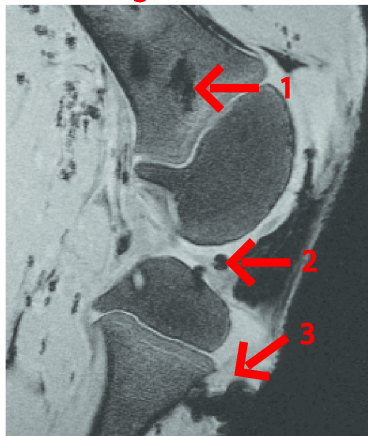
D.2.3 Eliminating artifact

For both knees, the joint was flushed with saline to eliminate artifact. To accomplish this, an open incision was created medial to the patellar tendon, thus allowing direct access to the intra-articular space. While flexing and extending the knee, saline was used to directly flush the joints. After flushing, the intra-articular space was filled with saline to expel any air bubbles created during this process. The joint was then wrapped in a pressure dressing to prevent the entrance of air bubbles, prior to the joints being re-imaged.

A. Intact



B. Passing Pin



C. Wash out

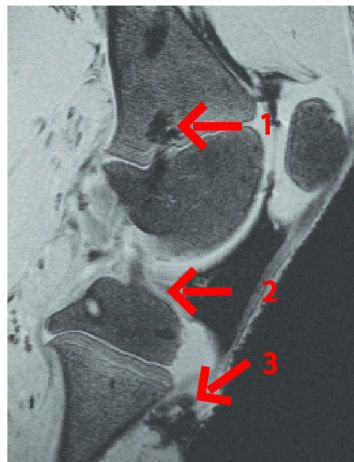


Figure D.2: Images of the first porcine knee. A) Intact showing 1. Bubble artifact in the musculature of the animal and 2. No artifact in the intra-articular portion of the joint. B. The joint following drilling with a passing pin. 1. Artifact clearly identified in the femoral tunnel, 2. Artifact identified in the intra-articular space, 3. Excessive artifact at the surface of the tibia's cortical bone where the passing pin tunnel started. C. The joint following washout and flushing. 1. Artifact still readily identified in one of the femoral tunnels, 2. Artifact in the intra-articular space is diminished, 3. Excessive artifact still identified at the tibial bone surface.

D.2.4 MR Imaging

All MR images were acquired using a surface knee coil on a 3T scanner (Siemens TIM Trio, Erlangen, Germany). A 3-D T1-weighted FLASH sequence (TR/TE/FA, 20/7.6/ 12°; FOV, 160 mm; matrix 512X512, slice thickness/gap, 1.5mm/0; avg 1; bandwidth, 130) was used. These MR images were inspected qualitatively to identify size and location of artifact.

D.3 Results

D.3.1 Intact

The first knee had no intra-articular artifact during the intact imaging, although some air bubble artifact was observed in the musculature and long bones of the knee (Figure 2). The second knee had two small isolated artifacts intra-articularly (Figure D.3).

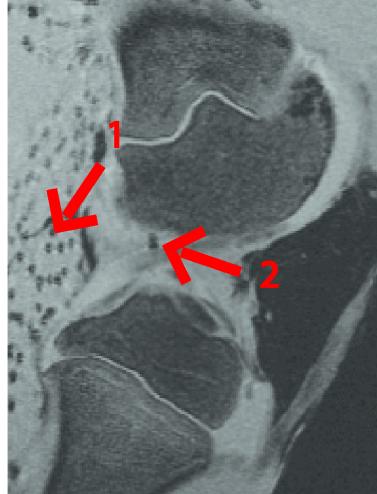
D.3.2 Passing pin

With the first knee, artifact was identified in all four tunnels drilled, with an excessive amount of artifact at the cortical bone surface where the passing pin entered the lateral tibia. Additionally, artifact was identified at the surface of the ACL intra-articularly (Figure D.2). With the second knee some artifact was identified in the four tunnels drilled, with an excessive amount at the cortical bone surface at the lateral tibia position. The second knee showed little to no intra-articular articular artifact (Figure D.3).

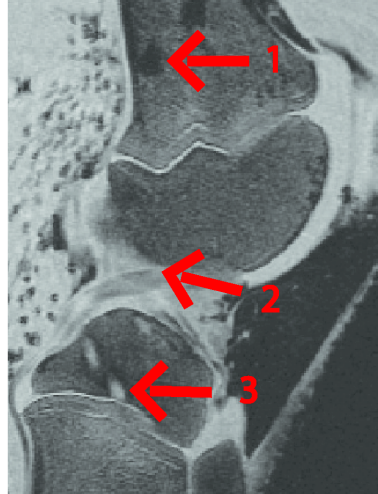
D.3.3 Cannulated drill bit

The second knee showed excessive artifact following drilling with the cannulated drill bit. Artifact was identified in the tunnels as well as clustered at the surface of the ACL and PCL (Figure D.3).

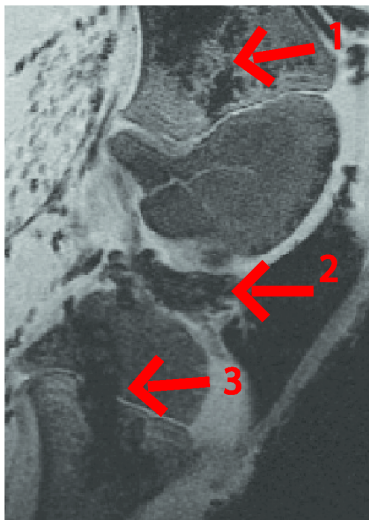
A. Intact



B. Passing Pin



C. Cannulated Drill



D. Wash out

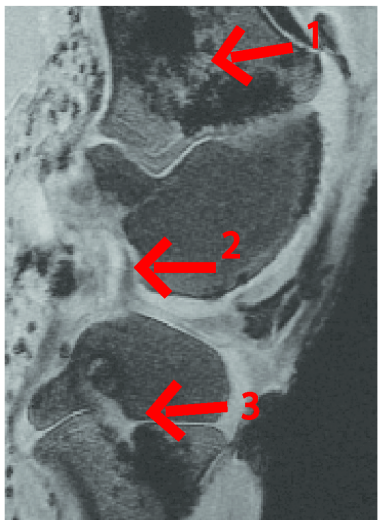


Figure D.3: Images of the second porcine knee. A) Intact knee showing 1. Bubble artifact in the musculature of the animal and 2. Air Bubble artifact in the intra-articular portion of the joint. B. The joint following drilling with a passing pin. 1. Artifact clearly identified in the femoral tunnel, 2. Air bubble artifact is no longer identified in the intra articular space, 3. No artifact in the tibial passing pin tunnels. C. The joint following tunnel creation with the cannulated drill bit. 1. Increased artifact in the femoral tunnel, 2. Excessive artifact intra-articularly in the area of the ACL and PCL. 3. Increased artifact in the tibial tunnel. D. The joint following washout and flushing. 1. Artifact still readily identified in one of the femoral tunnels, 2. Artifact in the intra- articular space is drastically diminished, 3. Artifact is still identified in the tibial tunnel but is diminished from the previous image.

D.3.4 Eliminating artifact

After the knees were flushed with fluid, the artifact clustered on the surface of the ACL and PCL was no longer identified (Figure D.3 and Figure D.3, respectively). Artifact in the tunnels and cortical bone may have been minimized slightly but was still apparent.

D.4 Discussion

Artifact was identified during tunnel creation and was related to a combination of collecting air bubbles and metal particulate. Artifact identified in the intact joints and the surrounding musculature indicates this artifact is not metallic and the result of air bubbles. Furthermore, the intact joints were minimally dissected prior to imaging, and when artifact was identified it appeared to cluster at the ACL and PCL surface. Dissection and removal of musculature from the long bones can leave a passage for air to enter the intra-articular space, especially at the patella-femoral groove. In other cadaveric studies that used a similar dissection technique, minor artifacts were also observed in intact joints.[1] Flushing the joint with saline and repeating the imaging process confirmed that these artifacts were due to air. After the joint was flushed, artifact due to air bubble that was aggregated on the surface of the ACL was no longer present, and was likely washed away by the use of saline. Concerns over air bubble artifact would be limited to *ex vivo* studies where the joint is dissected. For joints imaged *in vivo*, there should be minimal concern of air in the intra-articular space due to the living system maintaining homeostasis.

Metal particulate was also a cause of artifact with this pilot study. In the knees, artifacts were identified after the use of the passing pins and drill bits indicating that these artifacts were associated with metal hardware use. Additionally, the specific location and shape of artifact also implies metallic particulate (Figure D.2 C.3). Only a moderate ability to wash out artifact was identified. The best result of minimizing metal artifact was observed after a large volume of saline was used to flush the areas specific to the tunnels and intra-articular space along with repeated flexion extension of the joint.

Going forward, the best way to prevent artifact in an imaging study would be to include considerations for minimizing both air and metal artifact creation. Minimizing air artifact would be a primary concern for *ex vivo in situ* imaging where the joint has been removed from the animal and partially dissected. Leaving more musculature on the tibia and the femur will help keep the integrity of the joint capsule and prevent the entry of air. Alternatively, flushing the joint with saline helps to actively ensure the absence

of air bubbles following harvest. To help prevent metal artifact it may be best to flush the joint after the use of the passing pin as well as the cannulated drill bit, as they both create artifact individually.

D.5 Significance

Identifying the cause of artifact in an ACL reconstruction procedure and mitigating its effects could greatly minimize data loss and significantly lower cost in MR imaging studies.

D.6 Conflicts of interest

None.

D.7 Acknowledgements

Lynn Fannella at Brown Magnetic Resonance Facility (MRF) for assistance with image collection

D.8 References

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Appendix E

Volume and SI Predict Porcine Graft Laxity at 52 Weeks of Healing

Alison M. Biercevicz, Danny L. Miranda, Jason T. Machan,

Martha M. Murray, Braden C. Fleming

E.1 Objective

The purpose of this study was to determine if the same linear combination of volume and signal intensity (SI) used to predict graft or ligament structural properties in Specific Aim 1 could also predict AP knee laxity at 15 and 52 weeks of healing. We hypothesized that the combination of volume and SI would predict the graft or ligament AP laxity in a multiple regression model.

E.2 Methods and Statistics

The same reconstructed, repaired and transected knees used in the analysis of Specific Aim 1 were also tested for AP knee laxity before destructive structural properties testing. Similar to Specific Aim 1 the porcine knees were thawed and prepared for ex vivo AP laxity testing where the joint capsule was left intact unlike structural properties testing which requires the other supporting structures of the knee to be removed. With the joint capsule intact the AP laxity values for the knees were measured using a custom fixture with the knee fixed at 30°, 60°, and 90° flexion (AP30, AP60, AP90, respectively).[1,2] These fixtures are meant to mimic a clinical AP knee laxity test similar to clinical outcomes gathered in Specific Aim 6. Anterior- and posterior-directed loads of ± 40 N were applied to the femur with respect to the tibia by an MTS 810 Materials Testing System (MTS, Prairie Eden, Minnesota), while the AP displacements were measured. The AP laxity was reported as the displacement of the tibia with respect to the femur between the load limits of ± 30 N.[2] Both volume and SI were included in a multiple linear regression model to predict the knee AP laxity at 15 weeks and then at 52 weeks separately. The R-square values for both the 15 and 52 weeks models were reported as indicators of the strength of the relationships.

E.3 Results

Volume in conjunction with median SI, was able to significantly but not strongly predict AP30 at 15 weeks of healing; $R^2=0.16$ ($p=0.030$). Volume and SI were not able to predict AP60 or AP90 at 15 weeks; $R^2=0.13$ ($p=0.068$) and $R^2=0.06$ ($p=0.312$), respectively (Table E.1).

Volume in conjunction with median SI, was able to significantly predict AP30 and AP60 at 52 weeks of healing; $R^2=0.48$ ($p\leq 0.001$) and $R^2=0.56$ ($p\leq 0.001$), respectively (Figure E.1, Table E.1). Volume and SI were not able to predict AP90 at 52 weeks; $R^2=0.06$ ($p=0.483$) (Table E.1).

52 Weeks of Healing

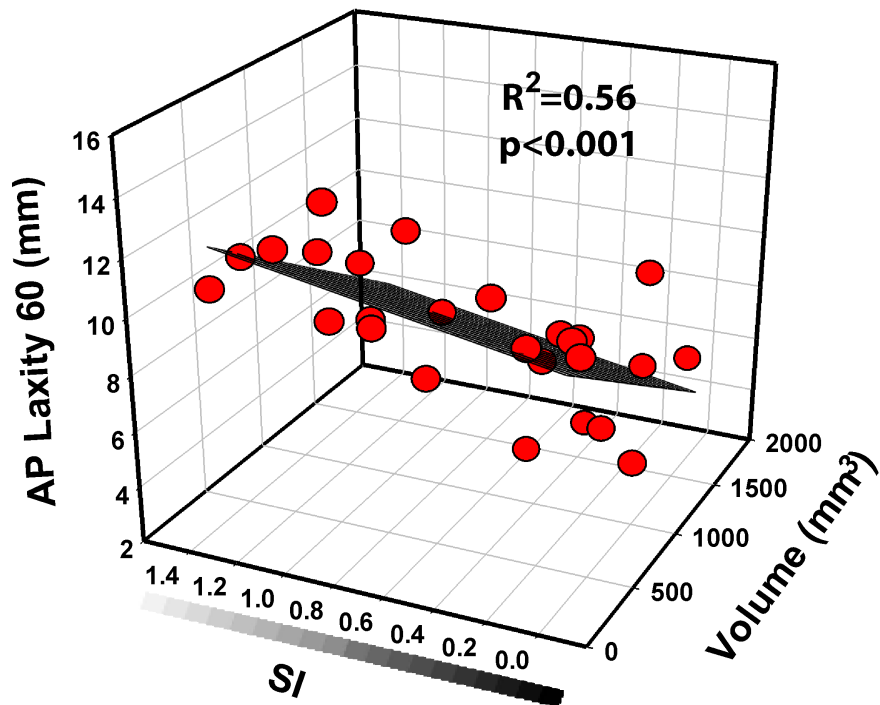


Figure E.1: The prediction plane for AP60 as a function of volume and of median SI for the reconstructed, repaired and transected ligaments at 52 weeks of healing.

Time Point	Dependant Variable	Independent Variable	R ²	p-value	Model p-value	Equation
15 Weeks	AP30	VOL	0.16	0.355	0.030	AP30 = 4.2 - (0.0008 X VOL) + (1.6 X SI)
		SI		0.025		
	AP60	VOL	0.13	0.03	0.068	AP60 = 10.8 - (0.002 X VOL) + (0.2 X SI)
		SI		0.759		
	AP90	VOL	0.06	0.129	0.312	AP90 = 8.9 - (0.001 X VOL) - (0.3 X SI)
		SI		0.731		
52 Weeks	AP30	VOL	0.48	0.006	<0.001	AP30 = 7.4 - (0.004 X VOL) + (1.0 X SI)
		SI		0.419		
	AP60	VOL	0.56	0.004	<0.001	AP60 = 10.8 - (0.004 X VOL) + (1.5 X SI)
		SI		0.188		
	AP90	VOL	0.06	0.249	0.483	AP90 = 11.0 - (0.002 X VOL) - (1.5 X SI)
		SI		0.335		

Table E.1: Summary of the reconstructed, repaired and transected ligament A-P laxity prediction equations for both the 15 and 52 week time points as a function of volume (VOL) and SI.

E.4 Discussion

In general higher volume and/or lower SI measurements were associated with lower AP knee laxity of the reconstructed, repaired and transected ligaments (Figure E.1). Prediction of graft or ligament AP laxity was stronger and more significant at 52 weeks of healing than at 15 weeks of healing. This difference in prediction strength between the two time points indicates that there may be a lag time before graft or ligament strength is reflected in laxity measurements. This finding mirrors results found with Specific Aim 6 where patient graft volume and SI were not predictive of AP knee laxity gathered with a KT-1000 at 3-year follow-up but was predictive of AP laxity at 5-year follow-up. Additionally, the combination of volume and SI was not able to predict AP90 at 15 weeks or at 52 weeks. This result was also supported by findings with previous animal studies, where AP laxity at 90 degrees of flexion was least indicative of reconstructed, repaired and transected ligaments health.[2,3]

It was found by combining the volume and SI values of the reconstructed, repaired and transected ligaments from Specific Aim 1 we were able to predict AP knee laxity at 52 weeks but not at 15 weeks of healing. This in combination with patient findings from Specific Aim 6 signifies that time after surgery may play an important role in determining how graft health affects knee laxity.

E.5 Conflicts of interest

None.

E.6 Acknowledgements

Braden Fleming and Martha Murray for the preceeding study involving a surgical animal model this analysis was done on.

E.7 References

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Appendix F

MR Variable Changes between Patient 3- and 5-year Follow-up for ACL Reconstruction

Alison M. Biercevicz, Braden C. Fleming

F.1 Objective

In Specific Aim 6, at 5-year follow-up, the MR variables of volume and median SI were able to significantly predict hop% and KOOS sub-scores. The volume and median SI prediction for APlaxity approached significance at 5-year follow-up. At 3 year follow up, these same MR variables were unable to predict APlaxity and KOOS sub-scores, but were able to significantly predict hop%. Additionally, we saw an increase in prediction strength between the 3- and 5-year hop% data. This difference in prediction strength between the 3- and 5-year follow-up indicates that time after surgery may play an important role in determining how graft structural properties affects surgical outcome.

The purpose of this study was to further evaluate the data from Specific Aim 6 by assessing the effect of time on patient graft MR and traditional outcome data. We tested the relationship between 3- and 5-year MR variables as well as the relationship between 3-year MR variables and future traditional outcomes at 5-year follow-up. We hypothesized that patient graft MR variables at 3-year follow-up would predict 5-year MR variables. Additionally, we hypothesized that MR variables at 3-years would predict future patient outcomes at 5-year follow-up. As a baseline comparison, the relationship between the 3- and 5-year MR variables of intact ACLs from a set of age matched control subjects[1] was also analyzed. For this control group we hypothesized that there would be no difference in the volume and SI MR parameters between the 3- and 5-year follow-up.

F.2 Methods and Statistics

The same MR variables and traditional outcomes from the patient population [1] used in Specific Aim 6 were also used for this analysis. First 3-year volume was used in a linear regression to predict 5-year graft volume (Figure F.1A). Then 3-year SI was used in a linear regression to predict 5-year graft SI (Figure F.1B). Next the 3-year SI was used to predict future patient traditional outcomes at 5-year follow-up (Figure F.2). The R-square values and p-values for the linear relationships were reported as indicators of the strength of the relationships. A group of age matched controls from the original patient study was also analyzed for time differences as a base line comparison, to the surgical group. A Repeated measures ANOVA was used to test for differences in intact ligament volume between 3- and 5-year follow-up and in intact ligament SI between 3- and 5-year follow-up for the control group.

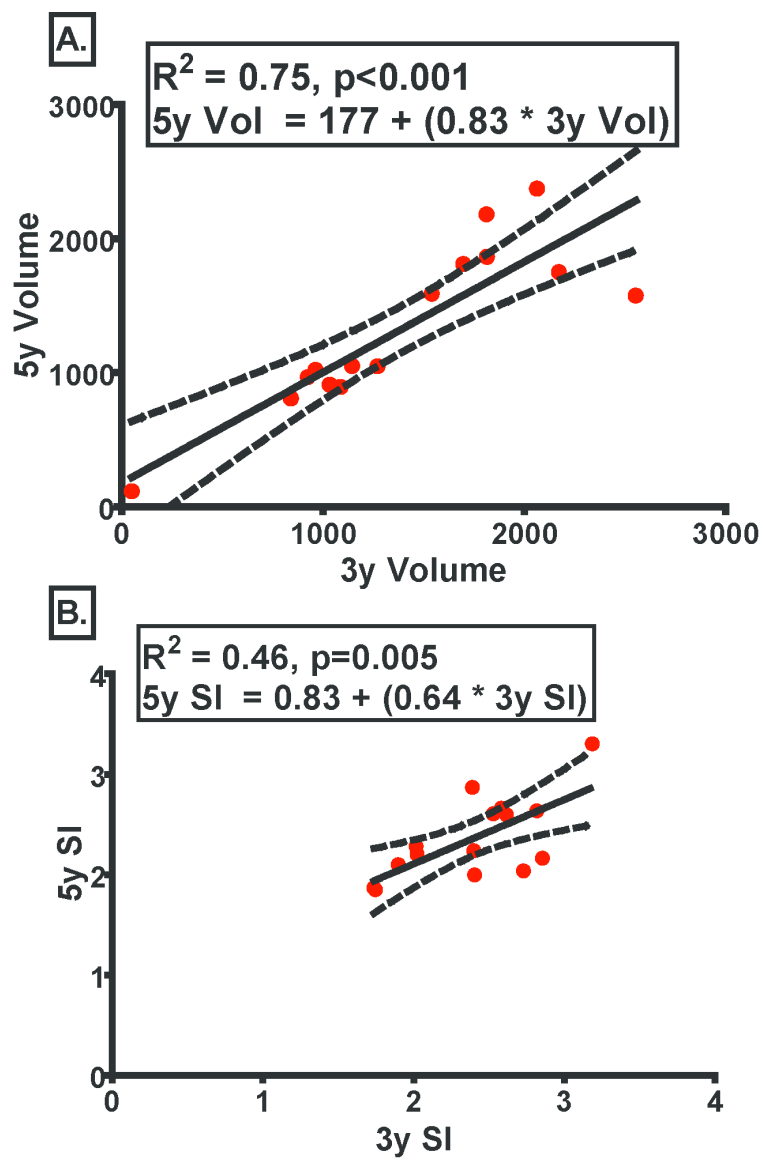


Figure F.1: The linear relationship of 3-year to 5-year patient MR variables. A) 3-year to 5-year volume and B) 3-year to 5-year SI.

F.3 Results

3-year graft volume was able to significantly predict 5-year volume; $R^2=0.75$ ($p<0.001$) (Figure F.1A). 3-year graft SI was able to significantly predict 5-year SI; $R^2=0.46$ ($p=0.005$) (Figure F.1B). 3-year graft SI was able to significantly predict the 5-year traditional outcomes of 5y Hop% and 5y KOOSqol subscore; $R^2=0.30$ ($p=0.03$) and $R^2=0.35$, respectively ($p=0.012$) (Figure F.2). No differences were found in volume or SI for the control group between 3 and 5 year follow up.

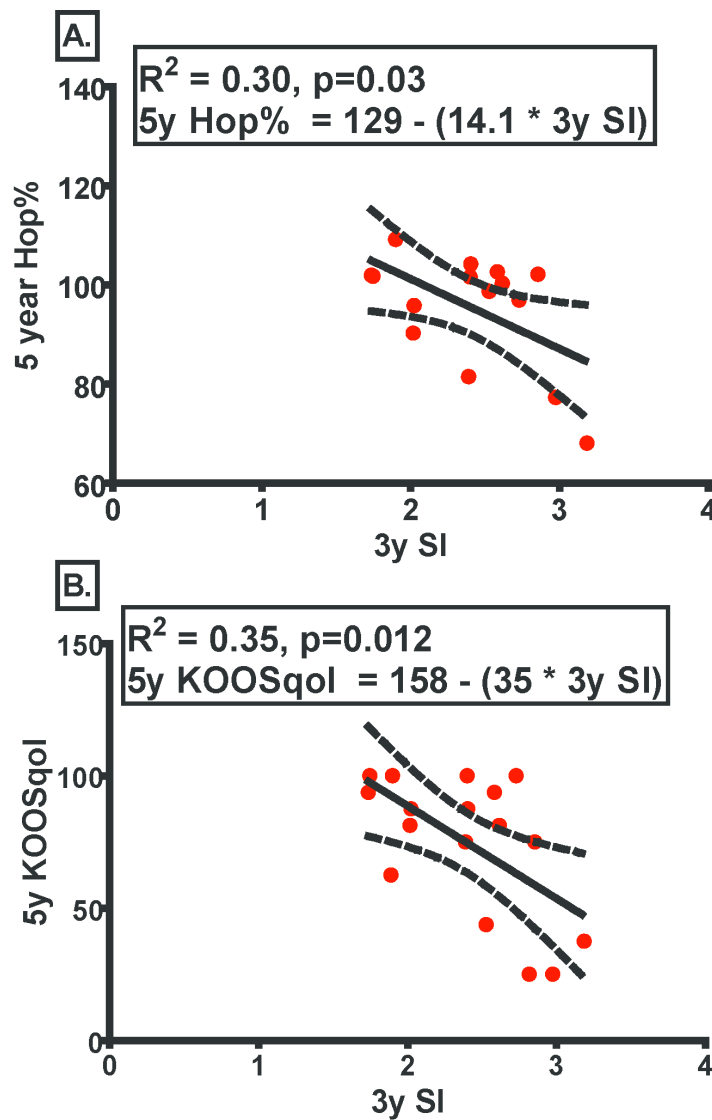


Figure F.2: The linear relationship of 3-year graft SI variables to 5-year traditional outcome. A) 5-year Hop% B) 5-year KOOSqol.

F.4 Discussion

As hypothesized 3-year graft volume predicted 5-year volume and 3-year graft SI predicted 5-year SI (Figure F.1). This indicates that the size and health of the graft at 3-year follow-up impacts the graft size and health two years later. Additionally, some patients had an increase in graft volume and SI, while some patients had a decrease in graft volume and SI between the follow-up times (Figure F.1). This could indicate that patient grafts are still changing or remodeling up to the 5-year time point. We found that there were no detectable differences in MR volume or SI between 3- and 5-year follow-up for the control group. The lack of change in MR variables for the control group further indicates that observed changes in the grafts of ACL reconstruction patients is likely due to graft remodeling.

We also found that early graft MR variables (3-year Volume and SI) could significantly predict later patient outcomes at 5-year follow-up (Figure F.2). This indicates that the health of the graft at earlier time points impacts later traditional outcome. This maybe particularly important if this relationship applies to even earlier time points. For instance if graft health in terms of MR variables at 1-year follow-up can predict later traditional outcomes at 5-years, this relationship could help influence clinical intervention for patients with poor MR outcomes at early time points.

In conclusion, the relationship of 3-year MR variables to 5-year MR variables and traditional patient outcomes may indicate that the graft continues to remodel, and signifies that time after surgery plays an important role in determining how graft structural properties affects traditional outcomes.

F.5 Conflicts of interest

None.

F.6 Acknowledgements

None.

F.7 References

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