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Greater anterior knee laxity (AKL) has been identified as a risk factor of anterior cruciate ligament (ACL) injury and could be indicative of a weaker ligament. While ACL morphometry and structural composition have the potential to affect ligamentous strength and resistance to external loadings, little is understood about how ligament size and structure may contribute to AKL. Thus, the purpose of this study was to determine the degree to which ACL morphometry and structural composition collectively predict AKL in active females and males. A cross sectional design recruiting active collegiate females and males were used. AKL was assessed by a knee arthrometer. T2-weighted magnetic resonance imaging (MRI) scans were utilized to obtain ACL morphometry as assessed by ACL volume, ACL width, and ACL cross-sectional area. T1-weighted MRI scans were utilized to acquire femoral notch width. Structural composition of the ACL was assessed by T2* and T2 relaxation times. All AKL and MRI measures were used to determine 1) sex differences in ACL morphometry; 2) sex differences in ACL structural composition; and 3) which ACL morphometric measure and MR relaxation measure were the strongest independent predictors of AKL and the degree to which ACL morphometry combined with ACL structural composition to predict AKL in active females and males. Twenty college-aged active healthy males (180 ± 0.1 m, 84.0 ± 10.9 kg, 23.2 ± 2.9 yrs) and twenty females (167 ± 0.1 m, 61.9 ± 7.2 kg, 21.3 ± 2.3 yrs) were measured for AKL and underwent MRI testing on the left knee. Results revealed that males had 30% larger ACL volume and 18% larger ACL width than females, with no sex difference in ACL cross-

sectional area. There were no significant differences between sexes in ACL structural composition as assessed via T2 and T2* relaxation times. ACL volume was the strongest morphometric predictor of AKL in both males and females. Smaller ACL volume and lower T2 relaxation times collectively predicted AKL in females (R^2=.68), whereas smaller ACL volume and higher T2* relaxation times collectively predicted AKL in males (R^2=.44). The primary findings collectively indicated that ACL morphometry and structural composition independently and collectively correlated with AKL. Further, females had smaller ACL morphometry than males with the ACL volume likely being the most appropriate measure of ACL size in studies of sex biases in ACL injury. Investigation of factors associated with the established risk factor of greater AKL could advance future prevention efforts to enhance ligament strength.

THE RELATIONSHIP OF ACL MORPHOMETRY AND STRUCTURAL COMPOSITION TO ANTERIOR KNEE LAXITY

by

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To my father, sister (佩瑩), and brother (偉民) without all of your support- I would have never been able to complete my PhD. Thanks for being such a loving family, who always supported me and believed in me. I hope you are so proud of me. My dear niece (涵甄), nephews (振皓) and (秉叡), you guys make family more fun and adorable. Mom, I knew you are always looking at me and never left. If I have any achievements in my life, that's all for you. You are always in the bottom of my heart. Love you.

APPROVAL PAGE

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CHAPTER I

INTRODUCTION

Statement of Problem

Anterior cruciate ligament (ACL) injury frequently occurs in active populations with around 70% of ACL injuries resulting from non-contact injury mechanisms (Boden, Dean, Feagin Jr, & Garrett Jr, 2000; Gianotti, Marshall, Hume, & Bunt, 2009; Hootman, Dick, & Agel, 2007). The primary function of the ACL is to prevent anterior displacement of the tibia relative to the femur (Butler, Grood, Noyes, & Zernicke, 1978) with secondary functions to protect against increased knee abduction and tibial rotation motions (Markolf et al., 1995). While knee stability during functional activity is provided passively by the ligaments and actively by the muscles around the knee, (Noyes, Grood, Butler, & Malek, 1980) it is possible that when there is a delay or an error in the neuromuscular control system, active restraint is insufficient and a greater relatively demand is placed on the passive restraints (Hashemi, Breighner, et al., 2011; Hewett, Paterno, & Myer, 2002). During this situation, the capability of the ligament to resist the external load is critical in maintaining ACL integrity. However, in vivo research is limited with regard as to how the intrinsic factors of the ACL may be related to ACL injury.

Clinically, ACL function is most commonly assessed by anterior knee laxity (AKL) testing (Butler, Noyes, & Grood, 1980). Greater AKL has been identified as a risk

factor of ACL injury (Myer, Ford, Paterno, Nick, & Hewett, 2008; Uhorchak et al., 2003; Woodford-Rogers, Cyphert, & Denegar, 1994). Specifically, a prospective study of college-aged military cadets reported a 2.7 times greater ACL injury risk with anterior knee laxity value ≥ 1 SD above the mean (Uhorchak et al., 2003). Additionally, a 1.3 mm side-to-side difference of anterior-posterior knee laxity prospectively resulted in more than a 3-fold greater odds of ACL injury (Myer et al., 2008). Collectively these reveal that greater AKL has a demonstrated association with increased risk of ACL injury.

Anterior knee laxity is commonly defined as the anterior displacement of the tibia relative to the femur under a fixed load. Greater anterior-posterior knee laxity was associated with lower failure load one year after ACL reconstruction surgery in a canine population (Beynnon et al., 1994). AKL measures in an intact animal ACL were significantly less than in those with a ruptured ACL (Lopez, Hagquist, Jeffrey, Gilbertson, & Markel, 2004). Additionally, 3D finite element modeling reported that greater PCL graft laxity was associated with lower graft strength (Lai et al., 2015). While these studies are limited to ligamentous grafts, they collectively suggest that greater knee laxity could be indicative of a weaker ligament. Thus, factors associated with lesser laxity have the potential to be related to ligamentous strength. A better understanding of the factors associated with stronger or less lax ligaments may be of benefit in ultimately reducing ACL injury incidence.

The orthopedic biomechanics literature has well established that greater cross-sectional area of connective tissue is generally associated with greater resistance to the displacement (Nordin & Frankel, 1989). Specific to the ACL, this concept would indicate

that greater ACL morphometry (i.e. larger size) would be associated with less deformation of the ligament under the fixed load, thus less AKL. While this theory is supported by animal research which reported total anterior-posterior translation of knee was associated with ligamentous cross-sectional area (R^2=0.86) after ligament reconstruction (Grood et al., 1992), the relationship between ACL morphometry as measured by ligament width and AKL in healthy humans was relatively weak compared to the previous animal study (R^2=0.22) (H.-M. Wang, Shultz, & Schmitz, 2015). Such differences in the relationships of laxity to ligament size are likely explained in part by the different morphometric measures used. While the most predictive morphometric ACL measure has yet to be established, other intrinsic factors contributing to stronger ligaments should also be investigated in vivo.

The strength of the ligament may not be fully represented by the ligamentous morphometric characteristics as ligaments with similar morphometry may have different material properties due to compositional differences. The primary compositional structures of ligaments include type I collagen, type III collagen, proteoglycans, elastin and water content (Culav, Clark, & Merrilees, 1999; Nordin & Frankel, 1989). Lower collagen density was associated with lower strain at failure of cadaver ACLs (Hashemi, Chandrashekar, Mansouri, Slauterbeck, & Hardy, 2008). Different collagen fibers have various diameters, which may affect restraint capacity (Liu, Yang, al-Shaikh, & Lane, 1995). Collagen fibril orientation was associated with the ability of the ligament to resist external forces (Quapp & Weiss, 1997). Small amounts of Type V collagen and proteoglycans could determine the structural make-up of the larger diameter collagen

fibers, which may impact collagen fibers integrity (Nakamura et al., 2000; Raleigh & Collin, 2012). These collagen factors thus have the potential to affect failure load. Such works collectively indicate that a well-structured and well-organized collagen network matrix is capable of resisting higher external loads, thus also being less lax.

To the date we understand very little about how the compositional differences and associated material properties of ligaments affect ligamentous function in-vivo. Recent advances in quantitative magnetic resonance imaging (MRI) have allowed insight as to the material properties of ligamentous tissue. T2 relaxation times is referred as the transverse relaxation rate (Chavhan et al., 2009). Shorter T2 relaxation times reflect denser collagen, more organized collagen ultrastructure, and less water content (Matzat, van Tiel, Gold, & Oei, 2013). T2 relaxation times is largely influenced by the presence of free water molecules, which slow down the loss of transverse magnetization (Matzat et al., 2013). Denser and more organized collagen matrix restricts the motion of water molecules, thus reducing free water molecules, and enhancing dipole-dipole interactions which shorten T2 relaxation times (Fullerton & Rahal, 2007; Matzat et al., 2013). Due to the influence of free hydrogen distribution association with MRI signal intensity decay, differences in collagen structure may be detected in vivo by T2 relaxation times.

T2 relaxation times have been utilized in an in vivo animal model to predict ligamentous function. Specifically, lower T2 relaxation times when combined with greater ACL volume were associated with lower anterior-posterior knee laxity of animal ACL grafts (Fleming, Vajapeyam, Connolly, Magarian, & Murray, 2011). Similar to T2 relaxation times, T2* relaxation times, which considers both of the spin-spin interaction

and the interaction with the magnetic field, has been utilized in an animal study of ACL grafts (Biercevicz, Murray, et al., 2014; Chavhan et al., 2009). T2* relaxation times was negatively associated with yield load of healing ACL grafts (Biercevicz, Murray, et al., 2014). These findings indicate that T2 and T2* relaxation times may both be capable of detecting ligament strength and thus have a potential relationship to AKL. However, while T2* relaxation time was independently associated with ligament strength (Biercevicz, Murray, et al., 2014), T2 relaxation time was not independently associated with ligament strength (Fleming et al., 2011). This suggests that T2* relaxation times may be more sensitive in detecting ligament composition associated with ligamentous strength than T2 relaxation times. Currently based on our knowledge, a direct comparison of the ability of T2 and T2* to predict ligamentous strength or AKL has not been performed. Additionally, while T2* relaxation times has been utilized in human cadavers (Biercevicz, Akelman, Rubin, et al., 2015), the results did not support previous T2*relaxation times association with ligamentous strength in vivo animal study (Biercevicz, Murray, et al., 2014). Given the limitation of the cadaver model in fully representing a true physiologic environment, further human in vivo investigation of the T2 and T2* relations to ligamentous function is needed.

While we have learned that AKL can be influenced by a multitude of factors including circulating sex hormones (Shultz, Kirk, Johnson, Sander, & Perrin, 2004; Shultz et al., 2010; Shultz, Wideman, Montgomery, Beasley, & Nindl, 2012), genetic factors (Bell, Shultz, Wideman, & Henrich, 2012; Silman, Day, & Haskard, 1987), and lower extremity alignment characteristics (Shultz, Dudley, & Kong, 2012; Shultz,

Schmitz, Nguyen, & Levine, 2009), the influence of morphometric and intrinsic factors of the ligament on in vivo AKL has received little attention. A better understanding of the relationship of morphometric and intrinsic factors to AKL may serve to inform future prevention programming to address the established risk factor of greater AKL by focusing on increasing ligamentous strength.

It is likely that relationships of ligamentous properties to AKL may be sex specific. ACL volume of female cadavers was smaller than in males (Chandrashekar, Slauterbeck, & Hashemi, 2005). Additionally, female cadaver ACLs had lower strain at failure (Chandrashekar, Mansouri, Slauterbeck, & Hashemi, 2006) and lower collagen density than males (Hashemi, Chandrashekar, Gill, et al., 2008). These findings indicated that ACL morphometry and structural composition associated with failure load could be sex specific. Because sex-specific hormones could mediate collagen turnover rate and impact ligament function (Shultz, Wideman, et al., 2012), the relationships of ACL volume and structural composition to AKL could differ between females and males. Due to disparities in sex-specific ACL structural composition and limited in vivo study of structural composition, further understanding the intrinsic factors on sex specific AKL could contribute the explanation of the higher injury risk in females (Beynnon et al., 2014).

Objectives and Hypotheses

Initial objectives are to 1) determine sex differences in ACL morphometry and femoral notch width (manuscript #1) and; 2) determine sex differences in ACL structural composition (manuscript #2).

- **Hypothesis 1:** Males will have greater ACL morphometry measures (ligament volume, width, and cross-sectional area) and wider femoral notch width than females.
- **Hypothesis 2:** Males will have shorter T2* and T2 relaxation times than females.

The next objectives are to determine which morphometric measure and which MR relaxation measure are the strongest independent predictors of anterior knee laxity (manuscript #3).

- **Hypothesis 3:** ACL volume will have greater predictive ability of anterior knee laxity than will ACL width and ACL cross-sectional area.
- **Hypothesis 4:** T2* relaxation times will have greater predictive ability of anterior knee laxity than will T2 relaxation times.

The primary objective of this investigation is to determine the degree to which ACL morphometry (as assessed by ligament volume, width, or cross-sectional area) and structural composition of the ACL (as assessed by T2* or T2 relaxation times) collectively predict to anterior knee laxity (AKL) in active females and males (manuscript #3).

- **Hypothesis 5:** The combination of smaller ACL morphometry (as determined from hypothesis 3) and longer quantitative MR relaxation time (as determined from hypothesis 4) will predict greater anterior knee laxity in males and females

Limitations and Assumptions

1. The findings from this dissertation may not be generalized to populations other than the physically active college-aged individuals.
2. The ACL volume measure requires manually segmenting the ACL contour from each MRI image. Depending on the field strength, chosen sequence, and individual participant variation, there is not a uniform resolution/pixel intensity distinguishing the ACL from surrounding soft tissues.
3. This work will control for femoral notch width because previous research reported that smaller femoral notch width area was associated with smaller ACL cross-sectional area (Dienst et al., 2007). There is a potential that other bony geometry may potentially affect ACL morphometry and structural composition.
4. A single tester will obtain all laxity and MRI measures; therefore prediction equations may not be generalizable to other testers.
5. The potential factors affecting structural composition of the ACL are not fully understood.
6. While clinically available, T2 and T2* mapping technology has not been widely used in human ligaments.
7. This work does not account for genetic factors that could impact structural composition of the ACL.
8. Even though we will control for phase of menstrual cycle in testing, the hormonal level within the 3 to 8 days of follicular phase can differ between participants (Landgren, Unden, & Diczfalusy, 1980).

9. Anterior knee laxity measure does not directly measure ACL function. ACL provides about 85 % of total anterior restraint with other passive structures providing less than 3% per structure (Butler et al., 1980).

Delimitations

1. Only healthy active males and females between the ages of 18 and 30 with no previous knee ligament injury and surgery will participate in this study
2. Previous studies reported that side-to-side measures of knee laxity and ACL volume demonstrated high degrees of symmetry (Jamison, Flanigan, Nagaraja, & W., 2010; Shultz & Nguyen, 2007). Bilateral knee laxity measures will first be used to determine the level of AKL symmetry. Then, AKL, ACL morphometry, and structural composition of the ACL will be only obtained from the left knee
3. All participants will be prohibited from engaging in strenuous physical activities for at least 24 hours before testing
4. All measures will obtained by a singer tester with established day-to-day reliability
5. While T2 and T2 * mapping will be utilized to assess the structural composition of the ACL, it is not a direct measure of collagen structure.
6. Data, results, and interpretation for females will be based on a limited window of the menstrual cycle (3 to 8 days)

Operational Definitions

Healthy: No history of injury or current chronic pain to the either lower extremity in the past 6 months that has resulted in limited physical activities; No previous history of significant injury to the capsule, ligament, or menisci of either knee; No previous history of the surgery to either knee.

Recreationally Active: An individual who currently engages in exercise at least 2 hours per week.

Adults: 18 to 30 years old.

T2 relaxation: T2 relaxation refers to the decay of transverse magnetization caused by the spin-spin interactions.

T2* relaxation: The time constant defining the loss of signal following excitation. Two components contribute to T2*. First, some signal loss occurs due to T2 relaxation. Second, some signal loss is caused by variation in precession angles for different spins within a voxel.

Radiofrequency Pulse: The electromagnetic pulse used in MRI to change the direction of the magnetic field.

Repetition time: The time from the application of an excitation pulse to the application of the next pulse.

Excitation time: The time of rotational magnetization out of alignment with the longitudinal axis, caused by the application of an RF pulse

Larmor frequency: The rate of precession of the magnetic moment of the proton around the external magnetic field.

B_0: The constant, homogeneous magnetic field used to polarize spins, creating magnetization.

Predictor Variables

ACL volume: The total volume of the ACL calculated from manual ACL segmentation of sagittal MRI images.

ACL Cross Sectional Area: ACL cross-sectional area calculated as the area of ACL segmented from the axial image.

ACL Width: ACL width as the width of a line transected the ACL, and was drawn perpendicular to Blumensaat's line.

Femoral Notch Width: Femoral notch width calculated at two-thirds of the notch depth with a line parallel to the ventral articular surface line.

T2* relaxation times: T2* relaxation times was generated from T2 relaxation image protocol of sagittal MRI images which indicate transverse relaxation rate. T2* does account for spin-spin interaction and magnetic field. Shorter relaxation times reflect more collagen density, organized collagen structure, and less water content (Matzat et al., 2013).

T2 relaxation times: Similar to the T2* relaxation times which was also generated from T2 relaxation image protocol of sagittal MRI images, but accounts only for spin- spin interaction of protons, not the interaction with the magnetic field.

Dependent Variable

Anterior Knee Laxity: The anterior displacement of tibia relative to the femur under 130 N force.

CHAPTER II

REVIEW OF THE LITERATURE

This review will address how a measure of ACL function (anterior knee laxity) may be associated with ligamentous intrinsic factors and how these factors may associate with ACL injury risk. Specifically, this review will address the current understanding of ACL epidemiology, function, injury mechanisms and ligamentous intrinsic factors that have been observed. Next, it will describe relationship of ACL morphometry and structural MRI composition measures associated to anterior knee laxity and ligamentous strength.

ACL Injury

Anterior cruciate ligament (ACL) injury has been stated as the "one of the major problems in sports medicine" (Renstrom, 2013). The ACL injury is commonly reported in the athletic population with around 0.11-0.17 injuries per 1000 Athlete-Exposures (Hootman et al., 2007). ACL injury has a collective health care cost in $4 billion in annual expenses (Brophy, Wright, & Matava, 2009; Gianotti et al., 2009; Griffin et al., 2000). Additionally, there are a long-term health burdens due to high rates of osteoarthritis development after initial ACL injury (Lohmander, Ostenberg, Englund, & Roos, 2004). The following section will address short-term and long-term effects of ACL injury.

Injury and Long-term Effects

Injury to the ACL impacts around 80,000 people annually in the United States with 70% of injuries in noncontact situations (Griffin et al., 2000). The recent costs of ACL injury are estimated at approximately $4 billion per year in the U.S. (Brophy et al., 2009). The injury compensation system in New Zealand reported that the expenses of ACL surgeries with following treatment costs involves in $11,157 per person (Gianotti et al., 2009). Across a 16 year injury epidemiology investigation of college athletes, the ACL injury rate was 0.15 per 1000 athlete-exposures with women's gymnastics (0.33), women's soccer (0.28), women's basketball (0.23) and men's spring football (0.33) being the highest risk sports (Hootman et al., 2007). Of approximately 80% of knee ligament surgeries and 65% of ACL surgeries, the patient was participating in sports activities at the time of injury (Gianotti et al., 2009). These reports collectively indicate that ACL injury results high expenses of health care and often occurs during sporting activity.

The ACL injury rate for 15 collegiate sports increased 1.3% on average per year from 1989 to 2004 (Hootman et al., 2007). Comparison of Belgian soccer teams between 2000 and 2010, revealed ACL injury rates decreased slightly (7%), but not significantly (Quisquater et al., 2013). Further, the rates of ACL reconstruction significantly increased during 1994 to 2006 from 32.94 to 43.48 per 100,000 person-years (Mall et al., 2014). These findings indicate that ACL injury continues to be a current health issue that is increasing in scope.

While the acute care and short-term postsurgical rehabilitation of ACL injury are significant issues, the long-term consequences are also considerable. A high rate of knee

osteoarthritis (OA) development and long-term knee functional limitations are related to ACL injury within 12 years of initial injury (Lohmander et al., 2004). Additionally, approximate 38% of ACL injuries are combined with medial meniscus tears (Frobell, Lohmander, & Roos, 2007). The combination of ACL and medial meniscus injuries significantly increased the risk of OA 15 years post-reconstruction (Cohen et al., 2007; Meunier, Odensten, & Good, 2007; Oiestad et al., 2010). Regardless of the patient had ligamentous reconstruction or meniscal repair surgeries; this may not reduce the incidence of knee OA development (Lohmander et al., 2004; Myklebust & Bahr, 2005). Numbers of adults over the age of 26 with knee OA symptoms have been estimated over than 9 million people with more than $185 billion in associated expenses in the U.S. (Kotlarz, Gunnarsson, Fang, & Rizzo, 2009; Lawrence et al., 2008). Collectively these results indicate that ACL injury not only has a short-term impact but also long-term negative effects on an individual's lifetime.

Summary

ACL injury is still a critical issue in sports medicine (Renstrom, 2013) with high costs (Brophy et al., 2009; Gianotti et al., 2009; Griffin et al., 2000) and the subsequent OA problems (Cohen et al., 2007; Lohmander et al., 2004; Meunier et al., 2007; Oiestad et al., 2010). Most ACL injuries are due to non-contact mechanisms in active populations (Boden et al., 2000). Due to the high ACL injury risk in active populations, understandings of how the ACL functions and how the ACL is strained during sports activity are needed to best design future prevention strategies.

Knee Function

Ligaments provide passive restraint to maintain knee stability (Noyes et al., 1980). Specifically, the ACL is the primary stabilizer in restraining anterior displacement of the tibia (Butler et al., 1980) with secondary functions to limit knee abduction and tibial rotation motions (Markolf et al., 1995). The following section will address the functionality of the knee joint and the role of the ACL in maintaining joint stability.

Knee Joint

The surface of the tibia relative to the femur permits three dimensional rotational and translational motions (Grood & Suntay, 1983; Pennock & Clark, 1990). The relative joint rotations between the femur and the tibia occur in the sagittal (flexion and extension) frontal (knee abduction and adduction), and transverse (internal and external rotation) planes (Grood & Suntay, 1983). The relative joint translations between the femur and the tibia occurs along the transepicondylar line (medial/lateral shift), along the floating axis (anterior/posterior drawer), and along the tibial long axis (compression/distraction) (Pennock & Clark, 1990). The rotational and translational motions in all three planes results in six degrees of freedom at the knee joint.

ACL Function

Knee ligaments provide the primary passive restraint for knee stability (Noyes et al., 1980). Specifically, the function of the ACL is to prevent the anterior tibial displacement relative to the femur (Butler et al., 1978) and to restrain tibial internal rotation and knee abduction (Markolf et al., 1995). In human cadaveric knees, performance of anterior drawer tests demonstrated that the ACL offers about 85% of the

total resisting force at 30 degrees of knee flexion with the other passive structures providing less than 3% per structure (Butler et al., 1980). During in situ anterior loading, the ACL provided about 82% of the total resisting force in 30 degrees of knee flexion and ACL strain gradually decreased as the knee was flexed (Takai, Woo, Livesay, Adams, & Fu, 1993). Further, the magnitude of the in situ ACL force was maximized at 15 degrees of knee flexion under 110 N anterior loads (Sakane et al., 1997). These findings indicate that passively prevention of anterior tibial translation is the main function of ACL and that its role is most critical in small knee flexion angles.

Multi-planar loading can also affect the ACL. 100 N of anterior force combined with 10 Nm of internal rotation or 10 Nm of abduction moments to the cadaveric knee lead to increasing ACL force in a small knee flexion angle (Markolf et al., 1995). Specifically, the ACL was strained maximally during combined anterior translation and internal rotation loads (Markolf et al., 1995). Thus the ACL has restraint roles in three anatomical planes.

Summary

The knee joint can be moved in three planes of six directions (Grood & Suntay, 1983; Pennock & Clark, 1990) with the primary function of the ACL being passive resistance to anterior tibial translation (Butler et al., 1980) and secondarily, to prevent internal and abduction motions (Markolf et al., 1995), especially in small knee flexion angles (Sakane et al., 1997; Takai et al., 1993).

Normal Ligament

Ligaments are composed of cellular material and extracellular matrix (C. B. Frank, Hart, & Shrive, 1999). These different structural components are necessary to maintain ligamentous function. The ligaments are viscoelastic structures with unique mechanical behaviors. The following section will describe ligamentous composition and mechanical behavior.

Ligament Structural Composition and Biology

Ligaments are bands which consist of relatively few cells and a large amount of extracellular matrix (C. B. Frank et al., 1999). The epiligament is a connective membrane covering the ligament that supports neurovascular structures and regulates water and metabolites (Chowdhury, Matyas, & Frank, 1991). Around two-thirds of the ligament is composed of water and collagen accounts for three-quarters of the dry weight (C. Frank, Amiel, Woo, & Akeson, 1985). More than 85% of the collagen is type I and less 10 percentage being type III. Other small proportions of the matrix are composed of elastin, proteoglycans, and glycoproteins (C. B. Frank, 2004).

Type I collagen is composed of two identical α1 chains and one α2 chain (Amiel & Nimni, 1993). Type III collagen consist of three identical chains and is abundant in blood vessels and is scant in the bone and ligaments (Amiel & Nimni, 1993). Comparing collagen ultrastructure, Type I collagen fibers are thick and dense, whereas Type III collagen fibers are thin and loose (Liu et al., 1995). Type I fibrils also have a relatively large diameter which could indicate that the ability to undertake higher mechanical loading (Culav et al., 1999). Type III is critical during the initial healing process and scar

tissue formation, thus could be an indicator of tissue maturity and offer the early mechanical support to the new synthesized tissue (Burgeson & Nimni, 1992). Type V collagen is a less abundant component of collagen fibrils, but plays an important role in the regulation of collagen fibril assembly and determination lateral collagen size make up which could affect collagen fiber integrity (Kadler, Baldock, Bella, & Boot-Handford, 2007). Type XII and XVI were fibril-associated collagens with interrupted triple helices which were found at the surfaces of Type I collagen and could interact with other collagen to impact ligament function (Kadler et al., 2007).

Compared to collagen content, smaller amounts of proteoglycans are detected in normal ligaments (C. B. Frank, 2004). The function of the proteoglycans is to bind together the collagen fiber and produce a gel-like material (Nordin & Frankel, 1989). The most predominant proteoglycan in normal ligamentous tissue is decorin (Plaas et al., 2000) with the amount of decorin negatively associated with the size of collagen fibrils. This indicates that proteoglycans may indirectly affect ligamentous structure and function (Nakamura et al., 2000). A large percentage of the extracellular ligamentous matrix consists of water content (C. Frank et al., 1985). The amount of water content could impact ligamentous biomechanical behavior and function. (Thornton, Shrive, & Frank, 2001). These findings indicate that amounts of proteoglycan and water content in extracellular matrix could be critical components with regard to ligamentous function.

Role of Intrinsic Factors on Ligament Function

Many intrinsic factors discussed above could impact ligamentous integrity and function. While most collagen fibers are arranged along the long axis of the ligament,

some collagen fibers are aligned in a nonparallel fashion which can assist against strains from different joint positions (C. B. Frank et al., 1999; Woo & Debski, 1999). The orientation of collagen fibers strongly affects ligamentous failure load. From cadaveric knee measurements, the ultimate load in the anatomical orientation to the ACL was 2160 ± 157 N which was different to the ultimate load in the tibial orientation (1602 ± 167 N) (Woo, Hollis, Adams, Lyon, & Takai, 1991). Specifically, the ability of collagen fibers to protect against a strain was higher in the longitudinal aspect (the force parallel to the fiber) than the transverse aspect (the force perpendicular to the fiber) (Quapp & Weiss, 1997). Thus a more organized, uniform collagen network may increase ultimate failure load of the ligaments.

Collagen concentrations may also affect ultimate failure load of the ligaments. Decreases in total collagen content are reported in injured ligaments (Amiel, Ishizue, Harwood, Kitabayashi, & Akeson, 1989). Specifically, the total collagen concentration from healthy ACLs was 80% of dry weight compared to 73.6% for partially torn ACLs (Amiel et al., 1989). Additionally, ACL failure load and failure strength in cadavers were highly correlated to percent area occupied by collagen (Hashemi, Chandrashekar, Mansouri, et al., 2008). Hence, collagen density is a critical factor impacting ligamentous integrity.

Individual structural components of the collagen fibers could also affect ultimate failure load of ligaments. Each type of collagen fiber has a different diameter and characteristics, which may influence the ability of resisting external forces (Culav et al., 1999; Liu et al., 1995). Smaller amounts of proteoglycans (Nakamura et al., 2000) and

Type V collagen (Raleigh & Collin, 2012) were negatively associated with collagen fibers diameters. Further proteoglycans make a gel-like material and contribute to the collagen fiber network integrity (Nordin & Frankel, 1989), thus helping to stabilize the extracellular matrix and increase resistance the deformation which may further influence ligament function and strength. Collectively, many ligamentous characteristics have the ability to affect ligamentous integrity and the associated failure load.

Ligament Biomechanics

The structural properties of ligaments are normally measured via tensile tests. The load-elongation curve is well described to characterize the behavior of the tissue (Korhonen & Saarakkala, 2011; Takeda, Xerogeanes, Livesay, Fu, & Woo, 1994; Woo & Debski, 1999). The typical load-elongation curve is upwardly concave at the beginning, but the slope is nearly linear before reaching the ultimate load. The specific shape of the curve depends on the properties and geometry of the ligament (Korhonen & Saarakkala, 2011; Takeda et al., 1994; Woo & Debski, 1999). The main structural properties assessed include linear stiffness, ultimate load, ultimate deformation, and energy absorbed at failure (Korhonen & Saarakkala, 2011; Takeda et al., 1994; Woo & Debski, 1999). The mechanical properties of the ligament are usually expressed in terms of stress-strain curve (Korhonen & Saarakkala, 2011; Takeda et al., 1994; Woo & Debski, 1999). Stress is defined as the applied force divided by the area of the ligament and strain is defined as the ratio of the change in length compared with the original length (Korhonen & Saarakkala, 2011; Takeda et al., 1994; Woo & Debski, 1999). The stress-strain curve is nonlinear which can be divided into several areas. (Figure 2.1) Region one is referred to

as the nonlinear toe region. The straightening of the crimp pattern in collagen will lead to the engaging of the ligament fibers until they reach their straightened condition. The second region is linear in which the collagen fibers are elongated. The slope of this region is called the Young's modulus. A steeper slope refers to greater resistance to deformation and could indicate that there is more collagen per unit area or could have larger fibril diameters. As the strain further increases, microtrauma will occur in the third region. Furthermore, increasing the strain will cause complete ligament rupture in the fourth region (Korhonen & Saarakkala, 2011; Takeda et al., 1994; Woo & Debski, 1999). The main parameters obtained from the stress-strain curve are the ultimate stress and ultimate strain (Figure 2.2) (Korhonen & Saarakkala, 2011; Takeda et al., 1994; Woo & Debski, 1999).

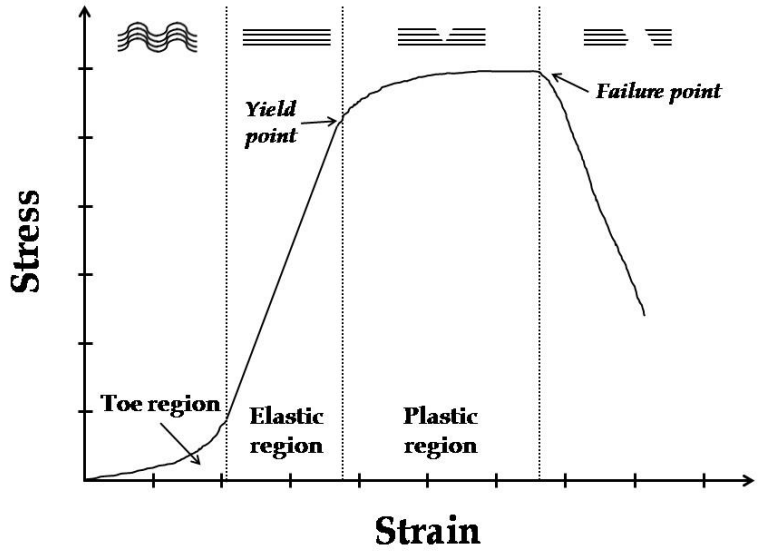

Figure 2.1 Stress-Strain Curve. Representative Stress-strain curve with labelled regions from ligamentous tensile testing. Collagen fibril structure is represented at the top of the graph (Korhonen & Saarakkala, 2011).

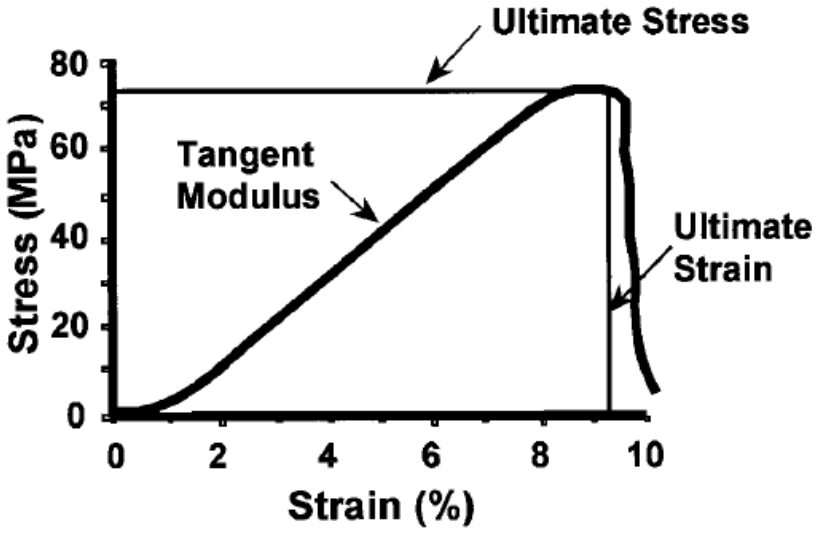

Figure 2.2 Stress-Strain Curve. Idealized stress-strain curve of ligamentous mechanical properties (Woo & Debski, 1999).

Ligaments display viscoelastic or time-dependent behavior under loading (Crowninshield & Pope, 1976; Fu, Harner, Johnson, Miller, & Woo, 1994; Woo & Debski, 1999). The area between unloading and loading curves from cyclic loading is called hysteresis which represents the energy loss from within the tissue (Woo & Debski, 1999). Creep and stress relaxation are two other types of behavioral characteristic of viscoelastic (Fu et al., 1994). Creep is a phenomena where a constant load is applied to the ligament and the deformation increases quickly at first, but then slowly progresses. Stress relaxation occurs when a constant elongation of ligament is maintained and the load decreases quickly at first, but then slowly decreases (Fu et al., 1994). Different loading rates may dictate different types of tissue damage. While slow loading conditions

have a higher chance to cause bony avulsion, faster loading conditions have greater probability to result in mid-substance failures (Crowninshield & Pope, 1976).

Ligament Physiology and Response to Mechanical Loading

Collagen turnover rate in a normal ligament is relatively slow in that the average half-life is about 300 to 500 days (Berger & Weiss, 2004). Exercise loading has been demonstrated to accelerate collagen synthesis (Kubo, Ikebukuro, Maki, Yata, & Tsunoda, 2012; Langberg, Rosendal, & Kjaer, 2001). Specifically, Tendon Type I collagen was significantly increased after two months of isometric training (Kubo et al., 2012). Another tendon training study reported that individuals involved in a military training program revealed an increase in Type I collagen turnover following one month of training (Langberg et al., 2001). Comparing the structural composition between ligaments and tendons reveals both of them are similar consisting of relatively few cells and a large proportion of extracellular matrix (Nordin & Frankel, 1989); hence, the training response of increase collagen turnover demonstrated in tendon may also apply to the ligament.

In a cell culture studies, mechanical loading resulted in increased levels of Type I and Type III collagen mRNA, increased fibroblast proliferation, elevation of growth factor expression, and increased enzyme activity (Hsieh et al., 2000; S. G. Kim, Akaike, Sasagaw, Atomi, & Kurosawa, 2002; Lin, Lee, O'Neal, McKean, & Sung, 1999; Mackey, Heinemeier, Koskinen, & Kjaer, 2008; Park et al., 2006; Zhou et al., 2005). These results indicate that mechanical loading modulates catabolic and anabolic collagen processes to maintain collagen function. In addition to collagen synthesis and breakdown, mechanical loading plays a critical role in allowing fibroblasts to maintain their ideal orientation. The

study reported that ACL fibroblasts align parallel to stretch direction which subsequently impacts the generation of an oriented collagen matrix (J. H. Wang, Jia, Gilbert, & Woo, 2003). These results indicated that mechanical load increases collagen synthesis and cell proliferation as well as manipulates collagen orientation, both of which are critical in maintaining the tensile properties and function of the ligament.

Exercise Loading and Ligamentous Properties

While mechanical loading is critical in maintenance of collagen integrity and function, ligamentous training studies are limited in number. An animal study reported that following a 5 week running protocol, the running group had greater MCL failure loads than the non-running animals (Adams, 1966). An increase of ligamentous failure load after training could be associated with an accelerated collagen synthesis as well as an increase in ligament mass, fiber diameter, and ligamentous cross-sectional area (Heikkinen & Vuori, 1972; Tipton, James, Mergner, & Tcheng, 1970; Tipton, Matthes, Maynard, & Carey, 1975). Further, from a human cross-sectional study, the size of the ACL in weightlifters was larger than the non-weight lifters' size after controlling for age, height, and weight (Grzelak, Podgorski, Stefanczyk, Krochmalski, & Domzalski, 2012). These collective findings indicated that an increased tissue size and enhanced structural composition characteristics via training, may contribute to tissue strength. However, prospective training studies in humans are lacking, and the mechanisms through which training potentially increases ligamentous properties and geometry are unknown.

Summary

While Type I collagen is the most abundant collagen in human ligament (Amiel & Nimni, 1993), each type of collagen fiber (Culav et al., 1999; Liu et al., 1995), various proteoglycans (Nakamura et al., 2000), and water content (Thornton et al., 2001) combine to maintain ligament function. Ligamentous mechanical behavior is described by a stress-strain curve (Takeda et al., 1994; Woo & Debski, 1999). Mechanical load regulates collagen synthesis and break-down to maintain collagen integrity function (Hsieh et al., 2000; Zhou et al., 2005), which could contribute tissue strength via enhance tissue properties or increase tissue size (Tipton et al., 1970; Tipton et al., 1975). However, the mechanisms through which ligamentous properties and geometry may interact to affect ligamentous strength in vivo are unknown.

ACL Injury Risk Factors

ACL injury risk factors have been separated in groups such as biomechanical (Olsen, Myklebust, Engebretsen, & Bahr, 2004), hormonal (Slauterbeck et al., 2002; Wojtys, Huston, Lindenfeld, Hewett, & Greenfield, 1998), and anatomical (Chaudhari et al., 2009; Ireland, Ballantyne, Little, & McClay, 2001; Stijak, Herzog, & Schai, 2008; Whitney et al., 2014) injury risk factors. Despite extensive research, it is still poorly understood how one injury factor can interact with others to affect injury risk. The following section will highlight the previously identified injury risk factors and why a focus on laxity may advance our understanding of ACL injury.

Injury Rates Between Males and Females

Approximately 72% of ACL injuries are due to non-contact mechanisms with the most common activities associated with ACL injury being basketball, football, and soccer (Boden et al., 2000). When the ACL injury rate is accounted per 1000 exposures, the most high risk sports are men's spring football (0.33), female gymnastics (0.33), female soccer (0.28), and female basketball (0.23) (Hootman et al., 2007). The disparate ACL injury rates between males and females have been consistently reported (Arendt, Agel, & Dick, 1999; Arendt & Dick, 1995; Beynnon et al., 2014; Myklebust, Maehlum, Holm, & Bahr, 1998; Prodromos, Han, Rogowski, Joyce, & Shi, 2007). Specifically, after accounting for sport and competition level, female athletes are twice more likely to suffer a first-time ACL injury than male athletes (Beynnon et al., 2014). From a sports-specific meta-analysis, the ACL injury rate of soccer female athletes was 0.32 versus 0.12 per 1000 exposures for males and basketball females was 0.29 versus 0.08 per 1000 exposures for males (Prodromos et al., 2007). Similar results in an epidemiology study across five years reported that soccer and basketball female athletes have around 3 times greater ACL injury risk than males (Arendt & Dick, 1995). Prospective cohort study of female handball athletes demonstrated a significantly higher ACL injury rate (0.31 per 1000 player hours) than males (0.06 per 1000 player hours) with a majority of injuries occurring during non-contact mechanisms characterized by high speed and plant-and-cut movements (Myklebust et al., 1998). In high school soccer and basketball athletes, girls have 3 to 4 times higher rate of ACL reconstruction surgery than boys (Powell & Barber-Foss, 2000). Collectively, these finding consistently report that females have a 3 to 5

times higher ACL injury rate than males during sporting activities. However, the exact mechanism(s) that explain this sex bias are not completely understood.

Biomechanical Risk Factors

The majority of ACL injuries are due to a non-contact mechanisms during weight-bearing activities (Boden et al., 2000). Video analyses of actual ACL injuries revealed that ACL injury typically occurs in knee abduction combined with either internal or external tibial rotation at close to full knee extension during planting-and-cutting movements or other one-leg landing positions (Olsen et al., 2004). However, from this study, the internal-external forces and moments applied to the knee cannot be determined at the time of the injury.

Cadaveric knee studies demonstrated that anterior force combined with internal tibial rotational or knee abduction motion in an extended knee posture (Markolf et al., 1995), isometric quadriceps contraction (Renstrom, Arms, Stanwyck, Johnson, & Pope, 1986), and impulsive knee compression forces (Withrow, Huston, Wojtys, & Ashton-Miller, 2006) increases strain to the ACL. These results reveal that excessive anterior displacement combined with knee abduction and internal tibial rotation motion could increase the strain to the ACL and potential increase the risk to rupture the ACL.

High-risk movement patterns may influence injury risk -Upon initial foot contact during landing tasks, vertical and posterior ground reaction forces create an external flexion moment at the knee joint that requires counteraction by an internal knee extension moment generated from the quadriceps to control knee flexion as the body decelerates (B. Yu, Lin, & Garrett, 2006). Thus aggressive quadriceps contraction with the shallow knee

flexion (<30°) produces anterior shear forces, resulting in anterior tibial translation (ATT) which could potentially injure the ACL (DeMorat, Weinhold, Blackburn, Chudik, & Garrett, 2004). Further, during weight acceptance activities, greater axial compressive loads with the knee extended led to greater ATT, regardless of increased quadriceps and hamstring activation (Schmitz, Kim, & Shultz, 2010). Small knee flexion angle, increased quadriceps muscle force, and greater posterior ground reaction force, led to increased knee extension moment with increased ACL loading (B. Yu & Garrett, 2007).

Joint anatomy can also impact ACL loading. Joint compressive forces (JCF) are contact forces which act perpendicular to the medial/lateral tibial plateau. At near full knee extension position, the JCFs acting on the posteriorly sloped tibial plateau will lead to a shear component that induces ATT (Hashemi, Breighner, et al., 2011). This anterior shear component with steeper posterior tibial slope (Marouane, Shirazi-Adl, Adouni, & Hashemi, 2014) could potentially produce a larger shear forces resulting in a higher ATT (Hashemi, Breighner, et al., 2011), thus, rupturing the ACL.

Collectively, higher-risk movement biomechanics could contribute to the increased ACL loading such as knee position, ground reaction force, quadriceps muscle activation, and tibial slope which alone or combined together will affect how the ACL is loaded in more than one plane of motion. Although not the focus of the current investigation, understanding neuromuscular and biomechanical factors association with ACL function may contribute future prevention efforts to reduce high-risk movement patterns.

Hormonal Risk Factors

Due to increased incidence of ACL injury in female populations, (Arendt & Dick, 1995; Powell & Barber-Foss, 2000; Prodromos et al., 2007), hormonal risk factors of ACL injury have been investigated. A retrospective study reported that a greater number of ACL injuries occurred before or after 1 to 2 days after the onset of menses (Slauterbeck et al., 2002). Self-reported menstrual history data demonstrated that a significantly greater number of ACL injuries occurred in the ovulatory phase of the cycle and fewer injuries occurred in the follicular phase (Wojtys et al., 1998). However, a systemic review did suggest a consensus of which phase of menstrual cycle association with higher ACL injury rate due to accurately identify hormonal level during the injury(Hewett, Zazulak, & Myer, 2007; Vescovi, 2011). These reports indicate that ACL injury may be associated with menstrual cycle phase.

Mechanisms by which sex hormones may influence injury risk -Circulating sex hormones have been investigated for their profound effect on a variety of collagen tissues. Estrogen, progesterone, relaxin, and androgen receptors have been found in the cells of the ACL, indicating sex hormones may have an effect on ligamentous structure and composition (Dragoo, Lee, Benhaim, Finerman, & Hame, 2003; Hamlet, Liu, Panossian, & Finerman, 1997; Liu et al., 1996). A study collecting blood samples through menses, ovulation, early and late luteal phases of menstrual cycle demonstrated that CICP (C-Propeptide of Type I Procollagen) was reduced in early and late luteal days, indicating that the magnitude of fluctuations of sex hormone concentrations across the menstrual cycle were sufficient to affect collagen metabolism (Shultz, Wideman, et al.,

2012). An ACL cell culture study reported that increased levels of estrogen lead to decreased levels of fibroblast proliferation and type I procollagen synthesis (W. D. Yu, Panossian, Hatch, Liu, & Finerman, 2001). However, the doses of estrogen are mediated by increasing progesterone. Specifically, fibroblast proliferation and type I procollagen synthesis rise with increasing progesterone doses when estrogen levels are held at constant (W. D. Yu et al., 2001). Functionally, ligament had a lower failure load when exposed in estradiol than without estradiol (Slauterbeck, Clevenger, Lundberg, & Burchfield, 1999). Although the specifics of how the mechanism(s) of hormones impact structural composition of the ligament are little understood, these findings suggest that sex hormones could influence ACL metabolism and collagen synthesis which may indirectly affect ligamentous function. A better understanding how hormone factors affect ACL structural integrity could benefit sex-specific ACL prevention efforts. Additionally, in studies of ACL injury not directly assessing the role of circulating sex hormones, controlling for time of menstrual cycle may be a prudent step.

Anatomical Risk Factors

The bony anatomy has also been investigated in relation to ACL injuries. From case-control study, a decreased femoral notch width (odds ratio [OR], 0.70) and an increased in bony ridge thickness at the anteromedial outlet of the femoral notch (OR, 1.61), were independently associated with ACL injury risk (Whitney et al., 2014). Radiographic measurement reported that ACL injured patients had smaller notch width and notch indexes than healthy controls (Ireland et al., 2001). A meta-analysis also indicated that the intercondylar notch width was narrower on ACL-injured patients

compared to healthy individuals (Zeng et al., 2013). Further, a case-control study reported that the tibial slope of the lateral condyle was greater in ACL patients (7.5°) than controls (4.4°), indicating that greater lateral tibial plateau slope may be an injury risk factor of the ACL (Stijak et al., 2008). Although the multifactorial bony anatomy may be injury risk factors of the ACL, the mechanisms by which bony anatomy may affect injury risk of ACL are inclusion.

Mechanisms by which bony anatomy may influence injury risk –The skeletal morphometry also plays a role in ACL injury risk. From a cadaveric knee study, tibial slope was associated with peak ACL strain and peak anterior tibial acceleration during simulated jump landings (McLean et al., 2011). In human single leg land-and-cut tasks, lateral tibial slope was correlated with peak anterior knee reaction force (McLean, Lucey, Rohrer, & Brandon, 2010), thus potentially increasing ACL strain. Smaller femoral notch width was associated with smaller in vivo cross-sectional area of the ACL and ACL volume (Charlton, St John, Ciccotti, Harrison, & Schweitzer, 2002; Dienst et al., 2007). Smaller femoral notch width may limit the size of the ligament which could be associated with less restraint capacity, thus increase the risk of ACL. However, how bony anatomy profiles combine or independently associate with joint laxity and ACL injury risk is little understood. Bony anatomy risk factors may modify the ACL loading profile which may result in alterations in ACL morphometry (Charlton et al., 2002; Dienst et al., 2007; McLean et al., 2010; McLean et al., 2011). These non-modifiable injury risk factors could contribute towards screening in high risk populations.

Knee Laxity Injury Risk Factors

Greater AKL has been identified as an ACL injury risk factor (Branch et al., 2010; Myer et al., 2008; Uhorchak et al., 2003; Woodford-Rogers et al., 1994). Prospective study reported a 2.7 times greater risk when the AKL value was ≥1 SD above the mean and GJL composite score was ≥ 5 (Uhorchak et al., 2003). A 1.3 mm side-to-side difference of anterior-posterior knee laxity lead to a more than 3-fold greater odds of sustaining an ACL injury (Myer et al., 2008). Further, ACL injured patients were reported to display greater internal rotational knee laxity in their contralateral limb than controls (Branch et al., 2010). While multiplanar greater knee laxity has demonstrated an association with ACL injury risk (Branch et al., 2010; Myer et al., 2008; Uhorchak et al., 2003; Woodford-Rogers et al., 1994), measurement of AKL is the most common method to assess ACL function (Shultz, Houglum, & Perrin, 2005).

Mechanisms by which laxity may influence injury risk –As stated earlier, upon initial foot contact during landing tasks, multiple-factors contribute to an increase in ACL loading such as knee position, ground reaction force, and muscle activation. ACL injury occurs when the external loading exceeds the ligamentous failure load (Slauterbeck, Hickox, Beynnon, & Hardy, 2006). As previously mentioned, the functional purpose of the ACL is to avoid knee displacement anteriorly (Butler et al., 1978), as well as to protect against knee abduction and tibial rotation (Markolf et al., 1995). Co-contraction of the quadriceps and hamstrings provide active protective mechanisms to the knee (Wojtys, Ashton-Miller, & Huston, 2002). It is possible that when there is a delay in co-contraction or dysfunction of knee muscles, active restraint is insufficient and the

demands on the passive restraints increase. (Hashemi, Breighner, et al., 2011; Hewett et al., 2002). During this situation, a weaker ligament will have an increased likelihood of failure. Greater anterior-posterior knee laxity in animal grafts was associated with lower failure load (Beynnon et al., 1994). AKL of intact ACLs was less than ruptured ACLs (Lopez et al., 2004). Using a 3D finite element model, greater PCL graft laxity was associated with lower graft strength (Lai et al., 2015). Further, while sex hormones could affect collagen synthesis (Shultz, Wideman, et al., 2012), an animal study reported that ACLs exposed to relaxin resulted in increased knee laxity and structurally weaker ACLs (Dragoo, Padrez, Workman, & Lindsey, 2009), indicating that hormones could alter the mechanical properties of the ACL and further influence on ligamentous function. These collectively suggest that greater knee laxity could be indicative of a weaker ligament.

A natural anterior shift of the tibia relative to the femur occurs in a fully extended knee when transitioning from a non-weight bearing to weight bearing position, (Beynnon, Fleming, Labovitch, & Parsons, 2002; Fleming et al., 2001; Torzilli, Deng, & Warren, 1994). This anterior shift is restrained by the ACL in the normal knee during weight bearing (Fleming et al., 2001; Torzilli et al., 1994). ACL deficient patients had a more posterior tibial contact position which could contribute to increased anterior tibia translation and internal rotation of the tibia (Scarvell, Smith, Refshauge, Galloway, & Woods, 2005). Individuals who have lax ACLs may undertake the similar situation of the ACL deficient, which allows for a more anterior displacement of the tibia during weight bearing position. Hence, a lax ACL may potentially undergo larger strains during sports activity, thus increasing the risk of ACL injury.

Peak anterior tibial translation (ATT) was positively correlated with greater AKL in both females and males during a drop landing (Torry et al., 2011). Cadaveric simulations of landing demonstrated that greater AKL was associated with greater magnitudes of ACL strain during simulated landing (Kiapour et al., 2014). These findings combined with greater anterior-posterior knee laxity being associated with lower failure load in animal grafts (Beynnon et al., 1994) indicate that increased AKL may allow greater anterior displacement of the tibia relative to the femur upon weight bearing position and thus, could potentially rupture the ACL. Further, in ACL deficient knees, the peak value of the tibial acceleration was significantly greater than controls during heel strike (Yoshimura, Naito, Hara, & Zhang, 2000). This anterior tibial acceleration is associated with greater strain to the ACL during simulated landing (McLean et al., 2011) and creates higher forces to the ligaments (Solomonow, 2009). Greater tibial acceleration during early axial load with greater initial and lesser terminal anterior stiffness predicated an increase in anterior shear force (Schmitz, Sauret, & Shultz, 2013). Hence, greater magnitudes of AKL, thus greater magnitudes of ATT, could be associated with greater acceleration of the tibia which increases the opportunity to injure the ACL. Collectively, greater magnitudes of AKL could result in biomechanical alternations which potentially could increase the risk of injuring the ACL. Thus, a better understanding of contributors to laxity could positively affect prevention efforts related to higher ACL strain.

Factors Thought to Contribute to Increased Knee Laxity

While AKL has consistently been reported as a risk factor of ACL injury, it has received relatively little attention due to common consideration of it not being

modifiable. However there are multiple factors related to AKL that may be modified. Circulating sex hormones have been demonstrated to affect AKL and thus, must be considered a factor in terms of joint laxity (Shultz et al., 2004; Shultz et al., 2010; Shultz, Wideman, et al., 2012). Specifically, AKL measurements are significantly different between the follicular and the ovulatory phase and between the follicular and the luteal phase, indicating that AKL in women could related to sex hormone concentrations (Deie, Sakamaki, Sumen, Urabe, & Ikuta, 2002). Further, AKL increased 3 to 5 days after changes in estradiol, progesterone, and testosterone were noted which indicated that changes in sex hormones across the menstrual cycle mediate changes in AKL (Shultz et al., 2004). A systematic review study analyzed AKL at three different occasions during a menstrual cycle and reported that AKL was highest during days 10-14, then 15-28, and lowest from days 1-9, indicating that the menstrual cycle may have a significant effect on AKL (Zazulak, Paterno, Myer, Romani, & Hewett, 2006). Collectively, AKL could vary across the menstrual cycle which suggests a need to control for phase of menstrual cycle when studying AKL.

Genetic profiles could also impact joint laxity (Bell et al., 2012; Silman et al., 1987). Previous studies have reported that a familial predisposition to greater joint laxity is a heritable trait (Silman et al., 1987). Certain genotypes have been associated with greater magnitude of AKL (Bell et al., 2012) with the presence of the AA genotype having been correlated with greater magnitudes of AKL (Bell et al., 2012) in females. The basic collagen structure of the α1 and α2 chains is encoded by certain genetic profiles, thus genetic factors could affect collagen make up and may potentially impact

ACL function(Collins, Posthumus, & Schwellnus, 2010; Khoschnau et al., 2008; Posthumus et al., 2009; September, Schwellnus, & Collins, 2007). These findings suggest that genetics play a role in AKL phenotypes.

Postural/anatomical characteristics can also affect AKL. Lower extremity alignment measures have been related to AKL joint laxity (Shultz, Dudley, et al., 2012; Shultz et al., 2009). Collectively, females with a less anterior pelvic tilt, smaller tibiofemoral angle, larger GR, and larger navicular drop were associated with greater AKL (Shultz et al., 2009). Lower anterior pelvic tilt and greater hip anteversion, greater GR, and greater navicular drop were collectively associated with greater AKL in males (Shultz et al., 2009). While postural/anatomical characteristics are associated with AKL, the best anatomical predictors of AKL are uncertain. Further, how these anatomical factors may have a long term effect on the magnitude of ACL loading and thus contribute to increase AKL is unknown. Hence, the current investigation is not focused on postural/anatomical characteristics.

As stated previously, knee stability is provided by both passive and active restraint around the knee (Noyes et al., 1980). Even though joint laxity primarily is designed to assess passive restraint capabilities, active restraint from musculotendinous structures that cross the joint may influence measures of joint laxity. Greater frontal and transverse planes knee laxity were associated with less lower extremity lean mass (LELM), but not in the sagittal plane knee laxity (Shultz, Pye, Montgomery, & Schmitz, 2012). This indicted that the surrounding muscle mass of the knee may contribute to the joint laxity to some extent but further understanding how muscle characteristics which

could modify affect joint laxity and which may non-modify is also needed, but not the focus of the current investigation.

From previous findings, many factors could contribute to AKL such as circulating sex hormones (Shultz et al., 2004; Shultz et al., 2010; Shultz, Wideman, et al., 2012), genetics (Bell et al., 2012; Silman et al., 1987), lower extremity alignment characteristics (Shultz, Dudley, et al., 2012; Shultz et al., 2009), and muscle mass (Shultz, Pye, et al., 2012). While sex hormones and genetics profiles could alter mechanical properties and the potential to influence ACL function (Collins et al., 2010; Dragoo et al., 2009), it is unknown how these factors directly correlate with ligamentous strength. While AKL has been demonstrated as a prospective ACL injury risk factor (Myer et al., 2008; Uhorchak et al., 2003), understanding the intrinsic factors of the ligament (ligamentous morphometry and structural composition) which could be associated with ligamentous strength and the respective AKL may benefit future prevention efforts by enhancing factors that increase ligamentous strength and subsequently lower AKL.

Exercise Loading May Influence Anterior Knee Laxity

While athletes have less AKL than non-athletes (Huston & Wojtys, 1996; Medrano Jr & Smith, 2003), it is uncertain if some part of their training may have affected their innate laxity. There are limited reports of how chronic sporting activity may affect AKL. Participants who played either handball or volleyball had greater AKL, but not in basketball (Vauhnik et al., 2009). On the other hand, swimming and basketball athletes have less AKL than the recreationally active participants (Ng & Maitland, 2001). These findings suggest that type and amount of chronic loading may be associated with

lower AKL. Given lower anterior-posterior knee laxity is associated with higher failure load (Beynnon et al., 1994), chronic loading which strengthens ligamentous properties (Tipton et al., 1970; Tipton et al., 1975) may contribute to lower AKL.

As AKL has been established as an ACL injury risk factor (Myer et al., 2008; Uhorchak et al., 2003), it has the potential to be a targeted focus of prevention/intervention programs. One intervention study reported that 12 weeks of knee extensor open kinetic chain resistance training at loads of 2 sets of 20RM resulted in a reduction of AKL in the ACL-injured knee group compared to control groups (Barcellona, Morrissey, Milligan, Clinton, & Amis, 2015). The report supported the notion that chronic loading accelerated ligamentous remodeling thus lowered AKL (Hayashi, 1996). On the other hand, 3 months of passive anterior loading intervention did not change the AKL in healthy females (Vauhnik et al., 2015). Although the ability of training to affect laxity is not firmly established, such findings give a suggestion that long-term training may contribute to decreased anterior knee laxity. Before undertaking training studies, a first step is to be to understand which intrinsic properties of the ligament may be related to ligamentous strength/laxity. Once established, training programs could be directed on specific intrinsic goals.

Morphometry and Structural Composition of the ACL as Injury Risk Factors

Given the material property positive relationship of size to strength discussed above, there is a rationale to investigate the role of ligament size on risk of ACL injury. A prospective case-control study reported that smaller ACL volume was an independent predictor of ACL injury (Whitney et al., 2014). Additionally a case-control study

demonstrated that ACL injured participants had smaller ACL volume on their non-injured side than controls which also indicated that ACL volume could be an injury risk factor (Chaudhari et al., 2009). The established sex bias in ACL injury rates may also help us to understand the role of ligament size in injury risk. A cadaveric study reported that females had 10% smaller ACL length, 20% smaller minimum area, and 35% smaller ACL volume than males even after adjusting for body height and weight (Chandrashekar et al., 2005). In a comparison of 50 males and 50 females' high school basketball athletes, ACL cross-section area was smaller in females (7.6 m) than males (8.7 m) (Anderson et al., 2001). These findings indicate that smaller morphometry could be associated with less ligamentous restraint capacity which could potentially increase the risk of ACL.

The restraint capacity of the ligament may not be fully represented by the ligamentous morphometry. Ligaments with similar ligamentous morphometry may have differences in their material properties. Lower ACL fibril density in human cadavers was associated with lower strain at failure (Hashemi, Chandrashekar, Mansouri, et al., 2008). In addition, cadaveric studies have observed that females have 8.3% lower ACL strain at failure, 18% lower collagen density, and 22.49% lower modulus of elasticity when compared to males (Chandrashekar et al., 2006; Hashemi, Chandrashekar, Mansouri, et al., 2008). These findings indicate that ACL structural composition could be correlated with ligamentous restraint capacity which potentially affects ACL injury risk.

The Relationship of AKL to ACL Size and Material Properties

As stated previously, greater anterior-posterior knee laxity of animal ACL grafts has been associated with lower failure load (Beynnon et al., 1994) and greater graft laxity was associated with lower graft strength (Lai et al., 2015). These suggest that greater knee laxity could be indicative of a weaker ligament. It is well established in the orthopedic biomechanics literature that greater connective tissue morphometry is generally associated with greater resistance to deformation (Nordin & Frankel, 1989). This suggests that greater ligamentous morphometry (e.g. volume, CSA, width) will lead to less deformation at a fixed load. Specific to the ACL, this concept would infer that greater ACL morphometry would be associated with less anterior knee joint laxity. While this theory is supported by animal studies reporting total anterior-posterior translation of knee being positively associated with ligamentous cross-sectional area (R^2=0.86) after ligament reconstruction (Grood et al., 1992), the relationship between ACL morphometry as measured by ACL width to AKL in healthy humans was relatively weak compared to the previous animal study (R^2=0.22) (H.-M. Wang et al., 2015). Thus, the investigation of other intrinsic factors on AKL is needed.

The structural composition of the ligament may also associate with ligamentous strength and laxity. Lower T2* relaxation times, which represents collagen density (Nissi et al., 2006), collagen structure (Nieminen et al., 2001), and water content (Lusse et al., 2000), was associated with higher yield load of healing ACL grafts (Biercevicz, Murray, et al., 2014). Further, greater graft volume combined with lower T2 relaxation times was highly associated with greater failure load (increased predictability) (Fleming et al.,

2011). Such findings indicated that greater collagen density, more organized collagen structure, and less water content could be indicative of ligament strength. (Fleming et al., 2011) Collagen type (Liu et al., 1995), collagen orientation (Quapp & Weiss, 1997) collagen structure (Nakamura et al., 2000; Raleigh & Collin, 2012), and collagen density (Hashemi, Chandrashekar, Mansouri, et al., 2008) could also be associated with failure load. These collectively indicate that a dense and well organized structural composition of the ligament is capable of resisting higher external loads, thus likely resulting in lesser laxity. However, the relationship of the combined ACL morphometry and structural composition on the AKL is little understood in healthy humans.

Summary

Multiple ACL injury factors have been investigated. Biomechanical study has focused on how external loading strains the ACL during deceleration mechanisms (B. Yu & Garrett, 2007). Circulating sex hormones could affect collagen metabolism which in turn impacts AKL (Shultz, Wideman, et al., 2012). These injury risk factors could be associated with AKL and greater AKL has been consistently identified as an ACL injury risk factor (Uhorchak et al., 2003). Many factors could contribute to AKL such as hormones (Shultz, Wideman, et al., 2012), genetics (Bell et al., 2012), lower extremity alignment characteristics (Shultz et al., 2009), and muscle mass (Shultz, Pye, et al., 2012). However, there is little attention on the relationship of intrinsic ACL properties to AKL. While ACL volume is predictive of ACL injury risk, the relationship between ACL volume and AKL in humans is little understood. Further, while structural composition of the ligament could be associated with ligamentous failure load (Fleming et al., 2011),

knowledge of how structural composition relates to AKL is limited. A better understanding of how ACL intrinsic factors influence AKL could contribute to future interventions which focus on increasing ligamentous strength and corresponding decreased AKL.

In-vivo Assessment of ACL Morphometry and Intrinsic Properties

Magnetic resonance imaging (MRI) is the primary technology utilized to acquire in vivo ligamentous morphometry (Anderson et al., 2001; Chaudhari et al., 2009) and structural composition of the ligament (Biercevicz, Miranda, Machan, Murray, & Fleming, 2013; Biercevicz, Murray, et al., 2014; Fleming et al., 2011). The following section will introduce the ligamentous morphometry and structural composition MRI measurements.

Magnetic Resonance Imaging (MRI)

MRI is a non-invasive modality that produces diagnostic images without the use of radiation (Hendrick, 1994; Jacobs, Ibrahim, & Ouwerkerk, 2007; Pooley, 2005). MRI utilizes the magnetic properties of hydrogen atoms to detect magnetic resonance (MR) signal changes in order to create images (Hendrick, 1994; Pooley, 2005). MRI systems rely on three components which include the primary magnet, gradient magnets, and radiofrequency (RF) coils (Hendrick, 1994; Jacobs et al., 2007; Pooley, 2005). Normally, hydrogen atoms are aligned randomly in humans. When placed in a strong magnetic field (Bo), the hydrogen atoms become aligned in a parallel or anti-parallel fashion (Hendrick, 1994; Pooley, 2005). A greater proportion of protons aligning in a parallel manner than anti-parallel manner produces a net magnetic vector of the hydrogen atoms (Hendrick,

1994; Pooley, 2005). The main magnetic field (Bo) results in the proton spin (precession) which allows for proton excitation and corresponding measurement of MR signal (Hendrick, 1994; Pooley, 2005). Gradient coils are used to alter the primary magnetic field for localization of the MR signal in three directions (Jacobs et al., 2007). Lastly, radiofrequency coils are used to transmit RF energy which leads to decreased longitudinal magnetization resulting in net magnetization vector flips to the transverse plane (Hendrick, 1994; Pooley, 2005). While T1 relaxation times refers the recovery time of the longitudinal magnetization, T2 relaxation times indicates the decay of transverse magnetization (Hendrick, 1994; Pooley, 2005). Changes in MR signal (i.e. changes in proton spin) received by the RF coils are changed from the frequency domain to the time-amplitude domain to create diagnostics images (Hendrick, 1994; Pooley, 2005).

ACL Morphometry Imaging

Structural imaging of soft tissue is most commonly performed using T2 weighted MR sequences (Anderson et al., 2001; Davis, Shelbourne, & Klootwyk, 1999; Dienst et al., 2007; Fayad, Rosenthal, Morrison, & Carrino, 2008). Multiple measures, which include cross-sectional area (CSA), length, width, and volume of the ACL in either sagittal or coronal planes have been used to assess ACL morphometry (Anderson et al., 2001; Chandrashekar et al., 2005; Charlton et al., 2002; Chaudhari et al., 2009; Davis et al., 1999; Dienst et al., 2007; Fayad et al., 2008; Jamison et al., 2010; Simon, Everhart, Nagaraja, & Chaudhari, 2010; Whitney et al., 2014).

With regard to using a morphometric ACL measure as a representative measure of ACL function, there does not seem to be a gold-standard method in the literature as the

multiple methods all have a degree of uncertainty/error in the measure. Much of the reason for this is due to the methods by which the MR data are collected and how the ACL is visualized. CSA is first identified from a point from the oblique sagittal plane which is one third of total ACL length proximal to the tibial insertion. Then, the marked point is viewed from the oblique axial plane and CSA is segmented and calculated (Whitney et al., 2014). CSA measures rely on single oblique sagittal and oblique axial planar images. Thus, given the non-uniform 3 dimensional nature of the ACL, CSA may not fully represent ACL morphometry.

ACL width is another morphometric measure that has been measured from one single sagittal image as the linear distance crossing the ACL which is perpendicular to the Blumensaat's line (Anderson et al., 2001). While ACL width is also the result of a single planar measure that does not take into account the non-uniform 3D ACL form, the contrast of ligamentous tissue to surrounding tissue is slightly better than measures from the oblique planes used in ACL CSA measures.

Conversely, ACL volume measures use multiple sagittal images to fully measure the entire ACL anatomy. ACL volume is measured by manually segmenting the ACL area from each sagittal image and then calculating the ACL volume across multiple images. This may more fully model the three dimensional nature of the ACL (Chaudhari et al., 2009; Jamison et al., 2010; Whitney et al., 2014).

Two dimensional measures such as CSA and ACL width may offer a benefit to researchers from a time demand and potentially offer few chances to introduce error to the measure. The ACL volume measure is more time consuming and potentially may

increase measure error from multiple-images due to the need to segment multiple images. However it is important to note that while all 3 measures are reported in the literature, ACL volume is the only ACL morphometric measure that has been reported as a predictor of ACL injury risk (Whitney et al., 2014). Collectively, ACL volume may be a more appropriate measure to assess ACL morphometry, but the other measures may merit further investigation as to the most appropriate measure of ligamentous function and laxity.

The literature is inconsistent with regard to the relationship of ACL morphometry to body size (Anderson et al., 2001; Charlton et al., 2002; Chaudhari et al., 2009; Fayad et al., 2008; Jamison et al., 2010). One study measured healthy male and female participants and reported from a multifactorial model that height was the only significant predictor of ACL volume (Jamison et al., 2010). Another match-control study of male and female participants reported that weight was a significant covariate of ACL volume, but not height (Chaudhari et al., 2009). However, these studies did not provide details of the strength of relationships between ACL volume to height or weight. Due to the inconsistent findings, height and weight should both be considered in models predicting ACL volume.

In-vivo Assessment of ACL Intrinsic Properties

In-vivo assessment of ligamentous structural characteristics has been limited to different MRI techniques. These primarily include signal intensity, T2, and T2* relaxation imaging. Signal intensity is calculated by normalizing the desired voxel intensity value to femoral cortical bone intensity (Biercevicz, Akelman, Fadale, et al.,

2015; Biercevicz et al., 2013). Signal intensity is thought to be a potential surrogate measure of ligament strength (Biercevicz et al., 2013; Biercevicz, Murray, et al., 2014). Specific to the ACL, the median normalized grayscale intensity value has been used as an outcome variable associated with ligamentous strength (Biercevicz, Akelman, Fadale, et al., 2015; Biercevicz et al., 2013). While easily calculated, signal intensity technique is based on a simple voxel intensity comparison. The voxel intensity could vary greatly depending on a number of local environmental factors, MRI scan sequence parameters and scanner hardware. Thus, making comparisons across studies quite are difficult. Further, this structural image technique is not established to reflect differences in structural composition such as collagen density and free water molecules.

T2 relaxation refers to the decay of transverse magnetization caused by the spin-spin interactions (Chavhan et al., 2009). Specifically, the tilt of the proton from the longitudinal magnetization into the transverse plane via a 90°radiofrequency (RF) pulse results in a transverse magnetization (Chavhan et al., 2009). In the transverse plane, the transverse magnetization rotates at the Larmor frequency and creates an MR signal in the radiofrequency receiver coil (Chavhan et al., 2009). The transverse magnetization reaches a maximum magnitude when all of the protons are in phase and begins reducing in magnitude immediately as protons start to go out of phase (Chavhan et al., 2009). The transverse relaxation is referred to as the process of dephasing and a 37% reduction in the amount of transverse magnetization (Chavhan et al., 2009). The transverse relaxation rate is called T2 relaxation (Chavhan et al., 2009). While T2 relaxation considers only spin-spin interaction, T2* relaxation, which is similar to T2 relaxation, considers both spin-

spin interaction and the interaction with the magnetic field (Pooley, 2005). Local magnetization is not completely stable resulting in magnetic field inhomogeneity. Inhomogeneous magnetic field leads to single loss due to variation in precession angles for different spins (Jung & Weigel, 2013).Therefore, T2* is shorter than T2 relaxation. (Figure 2.3)

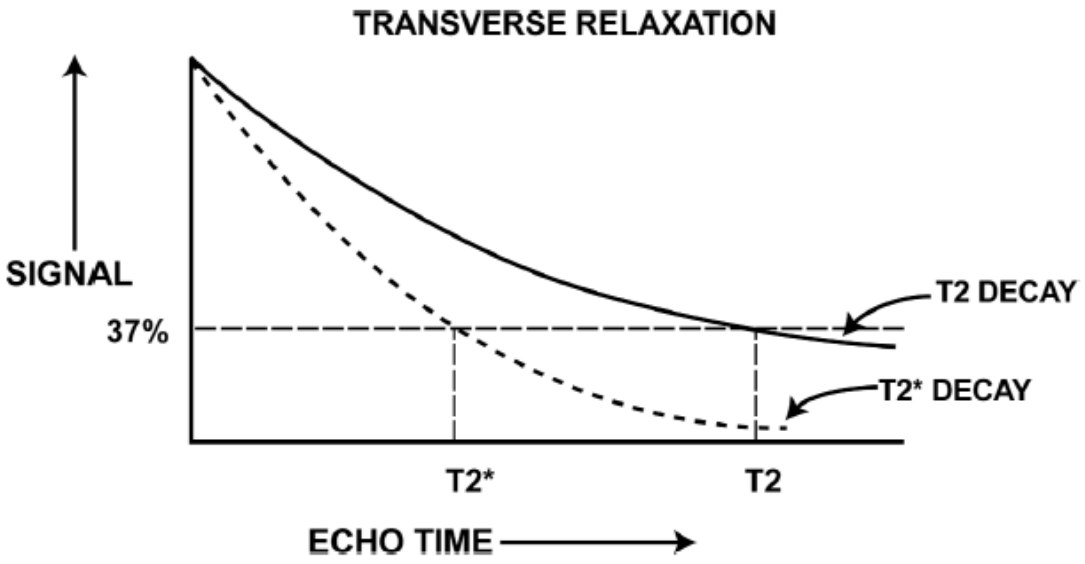

Figure 2.3 T2 and T2* Relaxation Curve. T2* relaxation is shorter than T2 relaxation (Chavhan et al., 2009).

The simplified T2 spin echo sequence consists of a 90° excitation pulse followed by a 180° refocusing pulse (Figure 2.4 and 2.5) (Jung & Weigel, 2013; Pooley, 2005). The time between the excitation pulse and the next peak echo is called echo time (TE) (Jung & Weigel, 2013; Pooley, 2005). Per the above statement, while T2 relaxation is the result of spin-spin interaction which is irreversible, T2* is the result of spin-spin interaction and the result of field inhomogeneity which can be reversed by application of

a 180° refocusing pulse (Jung & Weigel, 2013; Pooley, 2005). When the spin begins re-phasing and forming a new echo after application a 180° refocusing pulse, only signal intensity decay resulting from magnetic field inhomogeneity can be refocused (Jung & Weigel, 2013; Pooley, 2005). Thus, utilized spin echo sequence, the T2 relaxation can be determined by the peak amplitude of the echo (Figure 2.4) (Pooley, 2005).

(a) (b)

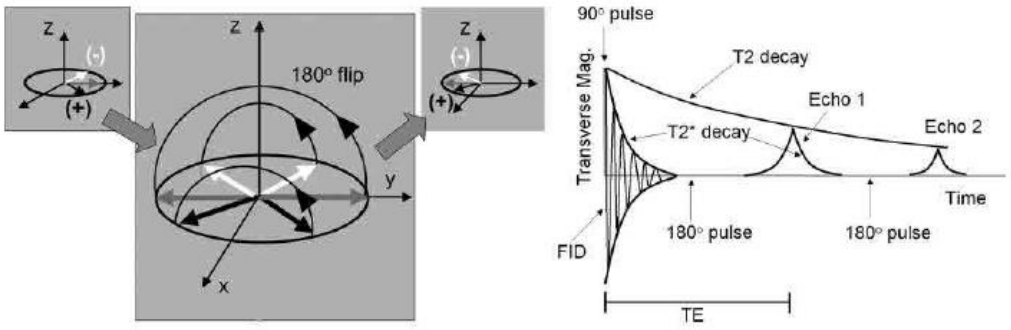

Figure 2.4 Spin Echo Sequence. (a) While protons begin to de-phase in the transverse plane following application of a 180° refocusing pulse, the proton spins will flip to the opposite axis which will allow the spins to re-phase and form the echo. (b) Spin echo sequence. After application a 90° excitation pulse, signal intensity decays immediately, which is T2* decay. After application of a 180° refocusing pulse, the spins will re-phase and de-phase again. The peak of the multi-echo will determined T2 decay (Pooley, 2005).

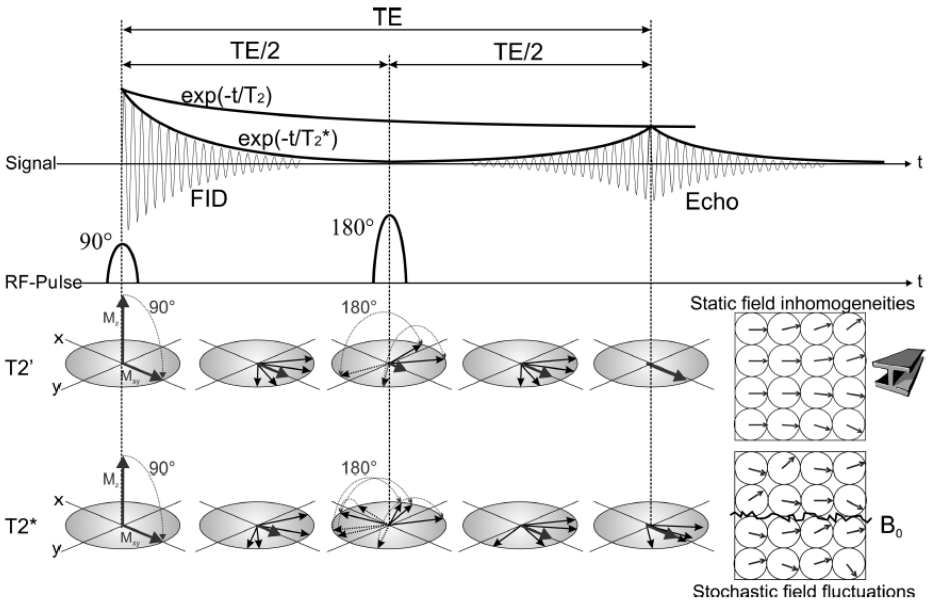

Figure 2.5 Spin Echo Sequence. The spins can be fully re-phased while considering magnetic field inhomogeneity which is described by T2'. T2* consists of on both T2' and T2 which signal intensity at TE is decreased (Jung & Weigel, 2013).

The gradient echo sequence consists of an exciting RF pulse with a flip angle less than 90°, but without a 180° RF pulse (Plein et al., 2011; Pooley, 2005). While, T2 relaxation cannot be produced without a 180° RF pulse, the gradient echo can create T2* relaxation by application of the gradient pulse which results in the de-phase and re-phase of the signal (Plein et al., 2011; Pooley, 2005). The T2* relaxation is determined by the peak amplitude of the gradient echo (Figure 2.6) (Plein et al., 2011).

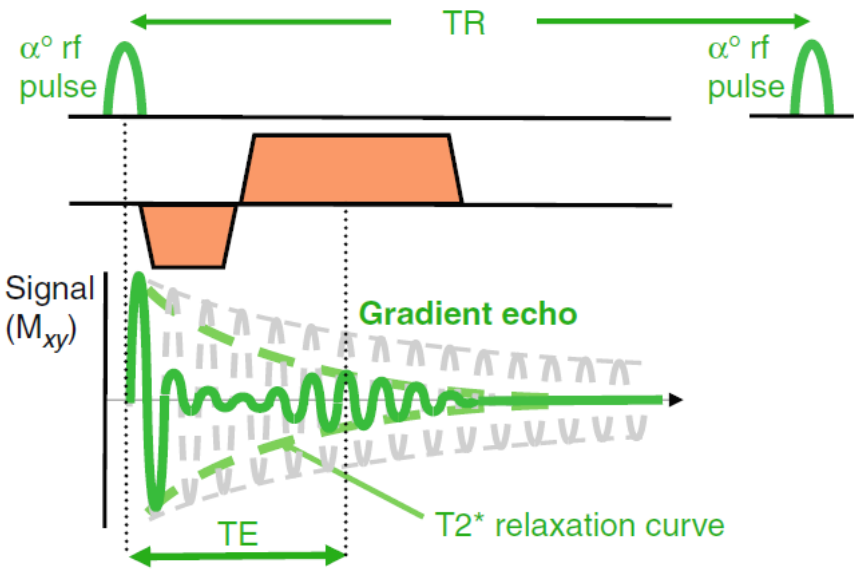

Figure 2.6 Gradient Echo Sequence. The gradient echo sequence consists of RF pulse with a flip angle α. The peak of the multi-gradient echo will determine T2* decay (Plein et al., 2011).

As stated earlier, T2 relaxation times is defined as the speed by which the proton loses phase coherence, following excitation. Due to the loss of coherence, an exponential decay of transverse magnetization will occur with a corresponding loss of MR signal. The rate of decay is largely influenced by the presence of free water molecules, which slow down the loss of transverse magnetization (Matzat et al., 2013). Relative to pathologic soft tissue, T2 relaxation of healthy soft tissue is shorter due to the collagen matrix's ability to trap and immobilize the protons of the water molecules. Conversely, longer T2 relaxation times is due to the degradation of the collagen matrix, which permits greater mobility of the water component of the extracellular matrix (Matzat et al., 2013). Moreover, the highly organized macromolecular matrix restricts the motion of water molecules and enhances dipole-dipole interactions, which shortens T2 relaxation times

(Fullerton & Rahal, 2007). Due to the significant influence of free water distribution on MR signal intensity decay, changes in collagen structure of the ligament may be detected by T2 relaxation times.

Shorter relaxation times could reflect more collagen density, organized collagen structure, and less water content, while longer relaxation times could reflect less collagen density, less organized collagen, and more free water content (Matzat et al., 2013). Specifically, T2 relaxation times can be modified by changing the water content, collagen fiber concentration, collagen orientation, and proteoglycan content (Li et al., 2011; Mosher, Dardzinski, & Smith, 2000; Regatte, Akella, Borthakur, Kneeland, & Reddy, 2002; Wayne et al., 2003; White et al., 2006). T2 relaxation times has been reported to be dependent upon the orientation of aligned collagen fibrils with respect to the main magnetic field (Bo) (Nieminen et al., 2001; Takeuchi, Sekino, Iriguchi, & Ueno, 2004; Xia, Moody, Burton-Wurster, & Lust, 2001). Further, in a study using MRI and polarized light microscopy, approximately 40% of depth-wise variation in T2 relaxation times was attributed to collagen fiber density (Nissi et al., 2006). A decreased collagen fiber density (Alhadlaq & Xia, 2004) and a reduced proteoglycan content (Wayne et al., 2003) has been shown to cause an increase in T2 relaxation times. The net orientation of the water molecules is also correlated with T2 relaxation times (Lusse et al., 2000). It is important to note that these findings indicating that T2 relaxation times is sensitive to the collagen characteristics and water content have been studied in articular cartilage. With regard to ligamentous tissue, T2 relaxation times is a feasible measure but quite limited with regard to its utilization in the literature.

Limited studies have investigated the relationship between MRI measures and ligamentous structural properties. Although the signal intensity method has been reported to be predictive of the biomechanical properties of the ligaments such as maximum load and yield load (R^2=0.37-0.42) in animals, signal intensity is sensitive to MRI parameters and scanner hardware (Biercevicz et al., 2013). Hence, the results may not be comparable across studies. Further, signal intensity reflects only a comparison between voxels, which may not represent intrinsic structural properties (Biercevicz, Akelman, Fadale, et al., 2015; Biercevicz et al., 2013).

T2 and T2* relaxation times both have the potential to assess ligamentous structural properties. Lower T2 relaxation times in combination with greater ACL volume was correlated with failure load (R^2=0.69) in animal ACL grafts (Fleming et al., 2011). Even though the sample size is relatively small (N=8), the strong relationship has been reported between the combination of ACL volume and T2 relaxation times to ligamentous strength (Fleming et al., 2011). Further, T2 relaxation times from this study was generated by multi-echo time (n=7) which has been demonstrated to be accurate and reduce sensitivity to noise (Biercevicz, Akelman, Fleming, Walsh, & Murray, 2014; Jenkins, Hickey, & Isherwood, 1987). Thus multi-echo T2 relaxation times could be associated with ligamentous strength. Further, lower T2* relaxation times was correlated with greater maximum and yield load of animal graft (R^2=0.78-0.93) (Biercevicz, Murray, et al., 2014). While T2* relaxation times was assessed by two gradient echo times, T2* relaxation times was still highly associated with ligamentous strength (Biercevicz, Murray, et al., 2014). ComparngT2* and T2 relaxation times studies reveals

that T2* relaxation times was independently correlated with ligamentous strength (Biercevicz, Murray, et al., 2014), with T2 relaxation times not independently associated with ligament strength (Fleming et al., 2011). These collectively findings suggest that both T2 and T2* relaxation times could potentially predict ligamentous biomechanical characteristics of ligament, but T2* relaxation times may be more sensitive in detecting ligament composition associated with ligamentous strength than T2 relaxation times. It is also important to note that the relationship of laxity to T2 and T2* relaxation times has not been established. However, neither T2 nor T2* relaxation times has been utilized in healthy human ligaments and reports of predictability between T2 and T2* relaxation times to ligamentous strength and strength are limited. Further there is no reports of the relationship of T2 and T2* relaxation times to clinical in-vivo measure of ligamentous function, such as AKL. A better understanding of the relationship of T2 and T2* assessment in human ligaments to AKL could advance ACL prevention efforts by focusing on which structural properties may be related to ligamentous laxity/strength.

Summary

While multiple MRI measures such as cross-sectional area (CSA), width, and volume of the ACL have been used to assess ACL morphometry (Anderson et al., 2001; Chaudhari et al., 2009; Jamison et al., 2010; Whitney et al., 2014), a gold-standard method which best represents of ligamentous function is uncertain. MRI techniques of T2 and T2* relaxation imaging to evaluate ligamentous structural characteristics have been demonstrated to predict in vivo ligamentous biomechanical characteristics in ACL grafts (Biercevicz, Murray, et al., 2014; Fleming et al., 2011). However, the predictability of

both T2 and T2* relaxation times to measures of ligamentous strength in healthy human ligaments is limited.

Summary

Anterior cruciate ligament (ACL) is a high expenses (Brophy et al., 2009; Gianotti et al., 2009; Griffin et al., 2000) with long term OA development issue (Cohen et al., 2007; Lohmander et al., 2004; Meunier et al., 2007; Oiestad et al., 2010). The primary function of the ACL is to prevent anterior tibial translation (Butler et al., 1980) and secondary functions are to protect internal and abduction motions (Markolf et al., 1995). Knee stability is maintained by passive restraints from the ligaments and active restrain from muscles (Noyes et al., 1980). While active restraint is insufficient due to dysfunction of the neuromuscular control system, the passive restraints from the ligaments are critical to maintain joint integrity (Hashemi, Breighner, et al., 2011; Hewett et al., 2002). During this situation, the strength of the ligament to resist the external load is critical.

Clinically, ACL function is measured by AKL (Butler et al., 1980) and grater AKL has been identified as a risk of ACL injury (Myer et al., 2008; Uhorchak et al., 2003; Woodford-Rogers et al., 1994). While hormones (Shultz, Wideman, et al., 2012), genetics (Bell et al., 2012), lower extremity alignment characteristics (Shultz et al., 2009) contribute AKL, little is attention on the relationship between intrinsic factors and AKL. Greater anterior-posterior knee laxity was associated with lower failure load and strength (Beynnon et al., 1994; Lai et al., 2015), indicating that greater AKL could be correlated with weaker ligament. While ACL volume and the structural composition of the ligament

were associated with ligament strength (Fleming et al., 2001; Hashemi, Chandrashekar, Mansouri, et al., 2008), how ACL morphometry combination with structural composition of the ACL relate to AKL is limited. A better understanding how intrinsic factors affect AKL could advance prevention efforts focused on enhance ligamentous strength.

While multiple MRI techniques have been utilized on ACL morphometry measures (Anderson et al., 2001; Chaudhari et al., 2009; Jamison et al., 2010; Whitney et al., 2014), a gold-standard measure is unsure. T2 and T2* MRI relaxation times has been used to assess ligamentous structural characteristics (Biercevicz, Murray, et al., 2014; Fleming et al., 2011), but the capability of these measures in vivo are limited. A better understanding gold-standard measure on ligamentous morphometry and structural composition could further advance measurement technique to detect ligamentous properties.

CHAPTER III

METHODS

The initial objectives are to determine sex differences in ACL morphometry, and femoral notch width and to determine sex differences in ACL structural composition. The next objectives are to determine which morphometric measure (as assessed by ligament volume, width, and cross-sectional area) and which MR relaxation measure (as assessed by T2* and T2 relaxation times) are the strongest independent predictors of anterior knee laxity. The primary objective of this research is to determine the extent to which ACL morphometry and structural composition of the ACL combine to predict to anterior knee laxity (AKL) in active females and males. The approach is to measure ACL volume, ACL width, and ACL cross-sectional area (CSA) as ACL morphometric variables and to measure T2 and T2* relaxation times as ACL structural composition variables and femoral notch width in active females and males, and to examine the extent to which these factors predict anterior knee laxity. The central hypothesis is that smaller ACL morphometry and longer quantitative MR relaxation times would predict greater AKL in male and females.

Participants

Forty (20 males, 20 females) healthy, recreationally active participants between 18-30 years of age, will be recruited from local universities to participant in this study. Healthy is defined as no history of injury or current chronic pain to the either lower

extremity in the past 6 months that has limited physical activities; no previous history of injury to the capsule, ligament, or menisci of either knee and no previous history of the surgery to either knee. Recreationally active is defined as an individual who current engages in exercise at least 2 hours per week. In order to control the hormonal influences on AKL, females will be based on a limited window of the menstrual cycle (3 to 8 days post menses onset). (Shultz et al., 2004) Inclusion criteria are: 1) current engagement in sport activities at least 2 hours per week; 2) no lower extremity injury in the last 6 months. Participants were excluded if they had: 1) previous history of injury to the capsule, ligament, or menisci of either knee 2) any vestibular or balance disorder 3) any metal or implanted medical device in the body. 4) do not meet predefined AKL criteria (Shultz et al., 2007) 5) cannot relax during the AKL measures All participants will read and sign an informed consent form approved by the University of North Carolina at Greensboro's Institutional Review Board for the Protection of Human Subjects. Each participant will attend a single testing session consisting of an anterior knee laxity assessment and MRI assessment. Participants will be instructed to avoid high intensity activities 24 hours prior to testing. All measures will be performed on the left knee. The activity rating scale (Marx, Stump, Jones, Wickiewicz, & Warren, 2001) (Appendix B) will be used to quantify participant activity level. Participants self-rated running, cutting, decelerating, and pivoting activities each as 0 (less than once per month), 1 (once per month), 2 (once per week), 3 (2–3 times per week) or 4 (4 or more times per week), resulting in a score from 0 to 16.

Procedures

The anterior knee laxity test will take place on the University of North Carolina at Greensboro's campus in the Applied Neuromechanics Research Laboratory. MRI scans will take place on the Joint School of Nanosciences & Nanoengineering which is 20 minutes away from UNCG campus. Upon arrival, participants will be provided written consent and will be assessed for anterior knee laxity measures. If the participants meet the anterior laxity screening criteria, demographics of age, sex, height, and weight are recorded. Next, participants will also complete physical activity and injury history questionnaires (Appendix A and B). After the completion of the questionnaires, participants are then underwent MRI examination.

Demographics and Questionnaires

Participants demographics of age, sex, height, and weight are recorded, and physical activity (type, duration, and intensity) and injury history are assessed by a standard questionnaire (Appendix A and B).

Anterior Knee Laxity Assessment

Anterior knee laxity (AKL) is defined as the anterior displacement of the tibia relative to the femur at 130 N load and will be measured by a single examiner using the KT-2000 Knee Arthrometer (figure 3.1) (Medmetric Corp, San Diego, CA). The subject will be placed in a supine position with the knee flexed 25 ± 5° over a thigh bolster. The foot/ankle will be rested in the foot cradle while a hook and loop strap is placed around both thighs. This method is used to prevent rotation of the lower extremities during testing. The examiner will first apply 90 N posterior-directed force then 130 N anterior-

directed force to the tibia, while displacement (mm) of the tibia with respect to the femur will be recorded by computer software. Three measures will be obtained and last two averaged for analyses. Anterior knee laxity (AKL) is defined as the average anterior displacement of the tibia relative to the femur over last two trials. The investigator has previously established between day measurement consistency and precision [ICC (SEM) =0.87 (0.5) mm] of this measure (Taylor et al., 2015). Potential subjects will be prescreened to obtain a wide distribution of AKL values in both sexes. This will be done to ensure equal amounts of average, above-average, and below average laxity within each sex. Previously reported data (Shultz et al., 2007) will be used to define average (M=5.6 ± 1.0 mm, F=8.1 ± 2.5 mm) above-average (>1 SD; M=6.6mm, F=10.6mm), and below average (<1 SD; (M=4.6mm, F=5.6mm) AKL.

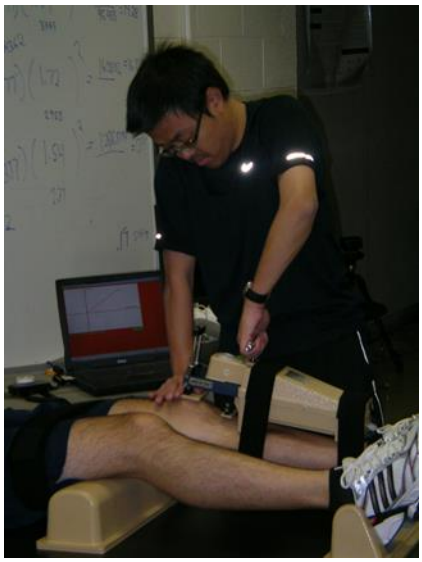

Figure 3.1 Anterior Knee Laxity Assessment. Anterior Knee laxity assessment which is obtained with KT-2000 arthrometer.

MRI Examination

MRI data will be acquired using a 3T Siemens Tim Trio scanner (Erlangen, Germany) and a 15 channel knee coil (Siemens Erlangen, Germany). T2-weighted, multiplanar MRI scans (repetition time (TR) =1300 ms; excitation time (TE) = 39 ms; Flip angle (FA) =160º; FOV=150x150 mm; voxel size = 0.5×0.5×0.5mm) will be used for ACL morphometric measures. T1-weighted, multiplanar MRI scans (repetition time (TR) =1200 ms; excitation time (TE) = 33 ms; FOV=160x160 mm; voxel size = 0.5×0.5×0.6mm) will be used for femoral notch width measures.

The T2 relaxation imaging will be performed using spin echo data sets with following parameters: repetition time (TR) =3040 ms; excitation time (TE) at 13.8, 27.6, 41.4, 55.2, and 69 ms; flip angle (FA) =180º; voxel size, FOV=160 x160 mm; voxel size= 0.4×0.4×3.0mm (Fleming et al., 2011). T2* relaxation will be performed using gradient echo data sets with following parameters: repetition time (TR) =1000 ms; excitation time (TE) at 8.26, 10.28, 12.3, 14.32, 16.34, 18.36, 20.38, 22.4, 24.42, 26.44, 28.46 and 30.48 ms; flip angle (FA) =90º; FOV=280 x280mm; voxel size = 0.5×0.5×3.0mm (Biercevicz, Akelman, et al., 2014; Biercevicz, Akelman, Rubin, et al., 2015). Full scan sequence details can be found in appendix C.

MRI Morphometric Data Reduction

ACL volume will be calculated as reported by Chaudhari et al (Chaudhari et al., 2009) using ITK-SNAP software (http://www.itksnap.org/pmwiki/pmwiki.php). ACL contouring of each sagittal slice will be done manually using a digitizing tablet (Wacom

DTK1300; Wacom Co, Kazo, Japan). All slices that will be contoured will be used in the calculation of ACL volume (Figure 3.2). The investigator has previously established intratester measurement consistency and precision [$ICC_{3,1}$ (SEM) =0.97 (36.1) mm^3].

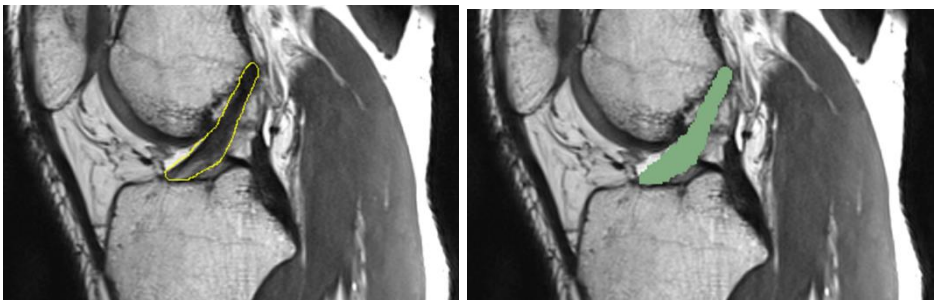

Figure 3.2 ACL Volume Measure. ACL was manually segmentation on each sagittal image. ACL volume then calculated from resultant ROI.

ACL width will be measured using Medical Image Processing, Analysis and Visualization software (MIPAV; http://mipav.cit.nih.gov) per methods described previously.(Anderson et al., 2001) First, the sagittal plane image that indicated the clearest image of Blumensaat's line will be selected. Blumensaat's line is the landmark which corresponds to the roof of femoral intercondylar notch as drawn on the sagittal knee joint image.(Seyahi, Atalar, Koyuncu, Cinar, & Demirhan, 2006) At the point of the notch outlet, ACL width will be determined by a line drawn perpendicular to Blumensaat's line that measured the distance across the ACL (Figure 3.3). The investigator has previously established intratester measurement consistency and precision [ICC (SEM) =0.98 (0.3) mm].

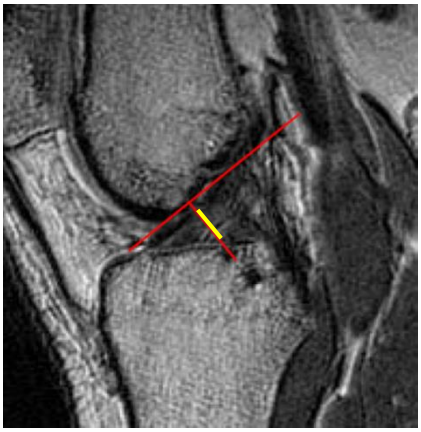

Figure 3.3 ACL Width Measure. ACL width was the distance across the ACL (yellow) on a line (red) perpendicular to the Blumensaat's line.

ACL cross-sectional area will be measured using ITK-SNAP software (http://www.itksnap.org/pmwiki/pmwiki.php) as reported by Whitney et al.(Whitney et al., 2014) From an oblique sagittal image perpendicular to the ACL, a point one third of the total ACL length from the attachment to the tibia will be identified (Figure 3.4a). After identification of this point, ACL cross-sectional area will be segmented and calculated from the oblique axial image (Figure 3.4b). The investigator has previously established intratester measurement consistency and precision [ICC (SEM) =0.87 (0.7) cm^2].

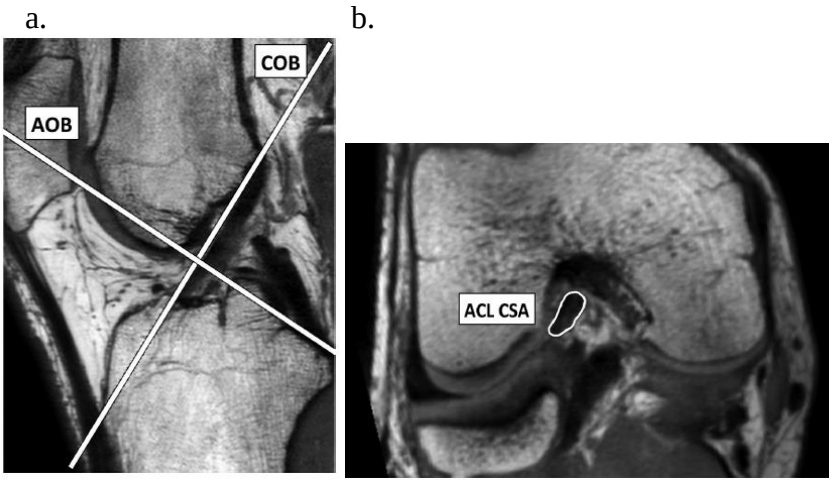

Figure 3.4 CSA Measure. (a) Identified CSA from the oblique sagittal image (b) CSA will be segmented and calculated from the oblique axial image (Whitney et al., 2014).

Femoral notch width will be measured using Medical Image Processing, Analysis and Visualization software (MIPAV; http://mipav.cit.nih.gov) as reported by Stein et al.(Stein et al., 2010) First, the clearest image of Blumensaat's line from the sagittal image and the beginning of the Blumensaat's line at the anterior outlet from the frontal image will be chosen to identify the axial image. Then, from the axial image, the articular surface line tangent to the medial and the lateral femur condyle will be drawn. Notch depth is the line perpendicular to the articular surface line. At two-thirds of the notch depth, the notch width will be calculated as the line parallel to the articular surface line (Figure 3.5). The investigator has previously established intratester measurement consistency and precision [ICC (SEM) =0.99 (0.2) mm].

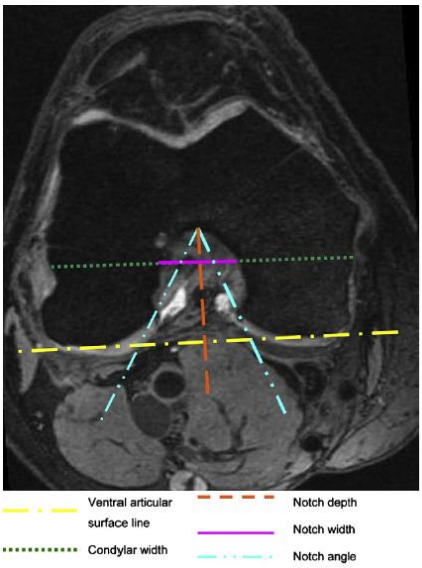

Figure 3.5 Femoral Notch Width Measure. Femoral notch width was the distance across the notch (purple) on a line (green) perpendicular to that line at 2/3 of the notch depth (orange) (Stein et al., 2010).

MRI Structural Composition Data Reduction

Using customized Matlab code (Mathworks Inc, U.S.A), the voxel-wise T2 relaxation maps will be calculated using the signal intensity (SI) relationship from all five echo times of the T2 relaxation imaging sequence. Equation: SI(TE)= So exp(-TE/T2), where SI(TE) are the voxel-specific SIs for the various echo times (TE) and where So is the signal intensity at the initial TE.(Fleming et al., 2011) To isolate ligament specific T2 values, the calculated T2 relaxation map will be registered to the structural imaging sequence using Slicer 3D Software (https://www.slicer.org/) and the mean T2 relaxation value of the voxels included in the 3D ACL volume described above will be calculated using ITK SNAP software (http://www.itksnap.org/pmwiki/pmwiki.php) and included in the analyses.

Using customized Matlab code (Mathworks Inc, U.S.A), the voxel-wise T2* relaxation maps will be calculated using the signal intensity (SI) relationship from all twelve echo times of the T2* relaxation imaging sequence. Equation: SI(TE)= So exp(-TE/T2*), where SI(TE) are the voxel-specific SIs for the various echo times (TE) and where So is the signal intensity at the initial TE.(Biercevicz, Akelman, et al., 2014; Biercevicz, Akelman, Rubin, et al., 2015) To isolate ligament specific T2* values, the calculation T2* relaxation map will be registered to the structural imaging sequence using Slicer 3D Software (https://www.slicer.org/) and the mean T2* relaxation value of the voxels included in the 3D ACL volume described above will be calculated using ITK SNAP software (http://www.itksnap.org/pmwiki/pmwiki.php) and included in the analyses.

Statistical Plan

Age, height (cm), mass (kg), AKL (mm), ACL volume (mm^3), ACL width (mm), ACL CSA (mm^2), femoral notch width (mm), T2 relaxation (ms) and T2* relaxation (ms) will be entered into Excel and transferred to SPSS for later analysis. The following statistical approaches will be used to test each of the following hypotheses.

Hypothesis 1: Males will have greater ACL morphometry measures (ligament volume, width, or cross-sectional area) and wider femoral notch width than females.

Hypothesis 2: Males will have shorter T2* and T2 relaxation times than females.

To test hypotheses 1 & 2, independent sample T-tests will be used to assess for difference in ACL morphometry, femoral notch width, and structural composition of the ligament

between males and females. Test variables will be ACL volume, ACL width and ACL cross-sectional area, femoral notch width, and T2 and T2* relaxation times.

Hypothesis 3: ACL volume will have greater predictive ability of anterior knee laxity than will ACL width and ACL cross-sectional area.

Hypothesis 4: T2* relaxation times will have greater predictive ability of anterior knee laxity than will T2 relaxation times.

To test hypotheses 3, separate sex-specific stepwise linear regression analyses will assess which ACL morphometry measure will be the strongest predictor of AKL. On the first step, femoral notch width will be initially entered to control for the relationship of ACL size and femoral notch width. (Dienst et al., 2007) On the next step, stepwise linear regression analysis will be used to assess which ACL morphometry variable (ACL volume, ACL width and ACL CSA) will be the strongest AKL predictor in males and females. The independent variables of the ACL morphometry regression will be ACL volume, ACL width and ACL CSA with femoral notch width as a control variable and with the dependent variable being AKL.

To test hypotheses 4, separate sex-specific stepwise linear regression analyses will assess which quantitative MR relaxation time will be the strongest predictor of AKL. The independent variables for the MR relaxation times regression will be T2 relaxation and T2* relaxation times with AKL serving as the dependent variable.

Hypothesis 5: The combination of smaller ACL morphometry (as determined from hypothesis 3) and longer quantitative MR relaxation time (as determined from hypothesis 4) will predict greater anterior knee laxity in males and females

To test hypotheses 5, separate sex-specific stepwise linear regression analyses will be tested the primary hypothesis that the ACL MR relaxation time and ACL morphometric measure, as determined from the hypotheses 3 & 4, will each have unique contributions in a multivariate model explaining the variance in AKL. The independent variables will be the ACL MR relaxation time and ACL morphometric measure as determined from the hypotheses 3 & 4 with the dependent variable being AKL. In the regression model, stepwise linear regression analysis will be used to assess the combined predictive ability of the included morphometric and relaxation time variables.

Power Analysis

All hypotheses will be evaluated at $p \leq .05$. Power analysis based upon hypothesis 1&2 revealed that a sample size of 16 (8 per group) would achieve 80 % power to detect a large effect size (d=1.52~2.13) using T-Testing with greater ACL volume, wider femoral notch width, and higher T2 relaxation times in males than females. Power analysis based upon hypothesis 3,4&5 revealed that a sample size of 36 would achieve 80% power to detect a large effect size of 0.30 attributed to the main 3 independent variables of interest for individual hypothesis 3,4&5 using F-Testing. A larger effect size of 0.30 was chose based preliminary data (N=10 with R-Squared of 0.32~0.68) using the dependent variable of AKL and the predictors of ACL volume, femoral notch width, T2* relaxation and sex. Based on the individual hypothesis 3, 4&5, selected predictors were chose for calculation effect size. To ensure adequate power for all variables, a sample size of 40 participants (20 males and 20 females) will be used.

CHAPTER IV

SEX SPECIFIC IN-VIVO ACL MORPHOLOGY

Abstract

Background: Females have consistently higher ACL injury rates than males. Reasons for this disparity are not fully understood. While ACL morphometric characteristics have an association with injury risk, little is known in vivo of sex comparisons on various ACL morphometric measures.

Hypothesis: Males have larger ACL volume, ACL width, ACL cross-sectional area. Males have larger ACL morphology after accounting for femoral notch width.

Study Design: Cross-sectional study

Methods: Magnetic resonance scans (3T) on the left knee were obtained from 20 active collegiate males and 20 active collegiate females. ACL volume, ACL width and ACL cross-sectional area measures were obtained from T2 weighted multiplanar images. Femoral notch width was measured from T1 weighted multiplanar MRI images. Independent sample T-tests examined sex differences in all ACL measures and femoral notch width.

Results: Males had larger ACL volume and ACL width than females. After controlling by femoral notch width, ACL volume was still greater in males than females.

Conclusion: Larger in vivo ACL morphometric measures were found in active healthy males than females. This suggests that ACL volume measure which is more

representative of ACL anatomy could be relevant in investigations of ACL morphometry and ACL injury risk.

Keywords: ACL volume, femoral notch width, MRI, knee

Introduction

Greater ACL injury rates in females have been consistently reported. (Arendt et al., 1999; Arendt & Dick, 1995; Beynnon et al., 2014; Myklebust et al., 1998; Prodromos et al., 2007) Even after accounting for sport and competition level, female athletes are twice as likely to suffer a first-time ACL injury compared to male athletes. (Beynnon et al., 2014)The ACL injury rate of soccer female athletes was 0.32 compared 0.12 per 1000 exposures for males, and basketball females was 0.29 versus 0.08 per 1000 exposures for males.(Prodromos et al., 2007) While this sex-bias in injury risk has been consistent, the exact reasons for this sex bias are not completely understood. From a structural perspective, it has been suggested that a smaller ligament size may be one factor that contributes to the higher rate of ACL in females. (Chandrashekar et al., 2005)

It is well established in the orthopedic biomechanics literature that as the size of connective tissue increases, it is generally associated with greater resistance to deformation. (Nordin & Frankel, 1989) Specific to the ACL, this concept would infer that larger morphometric characteristics of the ACL would be more capable of resisting external forces. This suggests that various measures of greater ligamentous size, such as volume, width, and cross-sectional area of the ligament, will result in greater ligamentous restraint capacity.

Thus, investigations of morphometric characteristics of the ACL may help to better understand risk of ACL injury. A prospective case-control study reported that smaller ACL volume was an independent predictor of ACL injury.(Whitney et al., 2014) An additional case-control study demonstrated that ACL injured participants had smaller ACL volume on their non-injured side than controls which also indicated that ACL volume could be an injury risk factor.(Chaudhari et al., 2009) The established sex bias in ACL injury rates may also help us to understand the role of ligament size in injury risk. A cadaveric study reported that females had smaller ACL volume and cross-sectional area than males even after adjusting for body height and weight. (Chandrashekar et al., 2005) In high school basketball athletes, ACL width and cross-sectional area were smaller in females than males.(Anderson et al., 2001) These findings indicate that smaller morphometric characteristics could be associated with less ligamentous restraint capacity which could potentially increase ACL injury risk. While multiple magnetic resonance imaging (MRI) measures, which include volume, width, and cross-sectional area (CSA) of the ACL, have been used to assess morphology of ACL, (Anderson et al., 2001; Chandrashekar et al., 2005; Charlton et al., 2002; Chaudhari et al., 2009; Davis et al., 1999; Dienst et al., 2007; Fayad et al., 2008; Jamison et al., 2010; Simon et al., 2010; Whitney et al., 2014) sex-specific in vivo comparisons of the various ACL morphometric characteristics are still limited.

Given the physical relationship of the ACL to the femoral notch and the potential for ligamentous impingement, (Fung & Zhang, 2003) the femoral notch is another anatomic factor that has been investigated with regard to ACL injury risk. A case-control

study reported that decreased femoral notch width was independently associated with ACL injury risk (odds ratio [OR], 0.70).(Whitney et al., 2014) Radiographic measurement of ACL injured patients revealed smaller notch width and notch indexes than healthy controls.(Ireland et al., 2001) Further, previous research reported that smaller femoral notch area was associated with smaller ACL cross-sectional area (Dienst et al., 2007) with females having smaller notch width index area than males.(Dienst et al., 2007) This suggests that smaller femoral notch width may limit the size of the ligament. This reduced ligamentous size could then be associated with less restraint capacity, thus increasing ACL injury risk.

Given that the in vivo sex-specific differences of ACL size and femoral notch width which may be corresponding to injury rate difference have not been fully established, the primary objective of the study was to determine sex differences in ACL morphometric characteristics and femoral notch width. It was hypothesized that males would have larger ACL morphometric characteristics and femoral notch width than females. A secondary objective was to determine sex differences in ACL morphology while accounting for femoral notch width. Better in vivo understanding of sex specific structural factors could contribute the explanation of the higher ACL injury risk in females.

Materials and Methods

Subjects

Healthy, recreationally active participants (20 males and 20 females) were recruited from local universities to participate in this study (demographics in Table 4.1).

Inclusion criteria were: 1) current engagement in sport activities at least 2 hours per week; and 2) no lower extremity injury in the last 6 months. Participants were excluded if they had: 1) previous history of injury to the capsule; ligament, or menisci of either knee; 2) any vestibular or balance disorder; and 3) any metal or implanted medical device in the body. All participants read and sign an informed consent form approved by the University of North Carolina at Greensboro's Institutional Review Board for the Protection of Human Subjects. Each participant attended a MRI testing session consisting of 3D T1 and T2 weighted magnetic resonance imaging (MRI) of the left knee. Participants were instructed to avoid high intensity activities 24 hours prior to testing. The activity rating scale (Marx et al., 2001) was used to quantify participant activity level. Participants self-rated running, cutting, decelerating, and pivoting activities each as 0 (less than once per month), 1 (once per month), 2 (once per week), 3 (2–3 times per week) or 4 (4 or more times per week), resulting in a score from 0 to 16.

MRI Examination

MRI data were acquired using a 3T Siemens Tim Trio scanner (Erlangen, Germany) and a 15 channel knee coil (Siemens Erlangen, Germany). T2-weighted, multiplanar MRI scans (repetition time (TR) =1300 ms; excitation time (TE) = 39 ms; Flip angle (FA) =160º; FOV=150x150 mm; voxel size = 0.5×0.5×0.5mm) were used for ACL morphometric measures. T1-weighted, multiplanar MRI scans (repetition time (TR) =1200 ms; excitation time (TE) = 33 ms; FOV=160x160 mm; voxel size = 0.5×0.5×0.6mm) were used for femoral notch width measures.

Morphometric Data Reduction

ACL volume was calculated as reported by Chaudhari et al (Chaudhari et al., 2009) using ITK-SNAP software (http://www.itksnap.org/pmwiki/pmwiki.php). ACL contouring of each sagittal slice was done manually using a digitizing tablet (Wacom DTK1300; Wacom Co, Kazo, Japan). All slices that were contoured were used in the calculation of ACL volume (Figure 4.1). For the establishment of intra-tester reliability and precision, ACL volume in 10 pilot participants was measured twice at least a week apart [$ICC_{3,1}$ (SEM) =0.97 (36.1) mm^3].

ACL width was measured using Medical Image Processing, Analysis and Visualization software (MIPAV; http://mipav.cit.nih.gov) per methods described previously. (Anderson et al., 2001) First, the sagittal plane image that indicated the clearest image of Blumensaat's line was selected. Blumensaat's line is the landmark which corresponds to the roof of femoral intercondylar notch as drawn on the sagittal knee joint image.(Seyahi et al., 2006) At the point of the notch outlet, ACL width was determined by a line drawn perpendicular to Blumensaat's line that measured the distance across the ACL (Figure 4.2). The investigator has previously established intratester measurement consistency and precision [$ICC_{3,1}$ (SEM) =0.98 (0.3) mm] of this measure.(H.-M. Wang et al., 2015)

ACL cross-sectional area was measured using ITK-SNAP software (http://www.itksnap.org/pmwiki/pmwiki.php) as reported by Whitney et al.(Whitney et al., 2014) From an oblique sagittal image perpendicular to the ACL, a point one third of the total ACL length from the attachment to the tibia was identified (Figure 4.3a). After

identification of this point, ACL cross-sectional area was segmented and calculated from the oblique axial image (Figure 4.3b). For the establishment of intra-tester reliability and precision, ACL cross sectional area in 10 pilot participants was measured twice at least a week apart [$ICC_{3,1}$ (SEM) =0.87 (0.7) cm^2].

Femoral notch width was measured using Medical Image Processing, Analysis and Visualization software (MIPAV; http://mipav.cit.nih.gov) as reported by Stein et al.(Stein et al., 2010) First, the clearest image of Blumensaat's line from the sagittal image and the beginning of the Blumensaat's line at the anterior outlet from the frontal image were chosen to identify the axial image. Then, from the axial image, the articular surface line tangent to the medial and the lateral femur condyle was drawn. Notch depth is the line perpendicular to the articular surface line. At two-thirds of the notch depth, the notch width was calculated as the line parallel to the articular surface line (Figure 4.4). For the establishment of intra-tester reliability and precision, femoral notch width in 10 pilot participants was measured twice at least a week apart [$ICC_{3,1}$ (SEM) =0.99 (0.2) mm].

Statistical Analysis

Independent sample T-tests examined the differences in ACL morphometry and femoral notch width measures between males and females. Secondarily we examined sex differences in ACL morphometry after normalization to femoral notch width. Test variables were ACL volume (mm^3), ACL width (mm), ACL cross-sectional area (cm^2), and femoral notch width (mm). The alpha level for all analyses was set priori at equal or

less than .05. All calculations were performed using the SPSS statistical software (version 21.0; IBM Corp, Armonk, NY).

Results

Sex-specific descriptive statistics for ACL volume, ACL width, ACL cross-sectional area and femoral notch width are shown in Table 4.1. Independent t-tests indicated that males had significantly larger ACL volume ($T_{.05\ (1,\ 38)} = -4.67$, $p < .001$), ACL width ($T_{.05\ (1,\ 38)} = -2.53$, $p = .016$), and larger femoral notch width ($T_{.05\ (1,\ 38)} = -5.52$, $p < .001$) than females. There was no sex difference in ACL cross-sectional area ($T_{.05\ (1,\ 38)} = -1.89$, $p = .067$). After normalizing by femoral notch width, males still had significantly larger ACL volume than females ($T_{.05\ (1,\ 38)} = -3.29$, $p = .002$). However, there were no sex differences in ACL width ($T_{.05\ (1,\ 38)} = -.61$, $p = .544$) and ACL cross-sectional area ($T_{.05\ (1,\ 38)} = -.26$, $p = .793$). The normalized ACL morphometric descriptives are shown in table 4.2.

Discussion

While a higher incidence of ACL injury in females has been repeatedly reported (Arendt et al., 1999; Arendt & Dick, 1995; Beynnon et al., 2014; Myklebust et al., 1998; Prodromos et al., 2007), the potential role of morphologic factors in ACL injury risk is little understood. (Whitney et al., 2014) In the current study we examined sex differences in anatomical factors to better understand the sex-bias in ACL injury. Our primary finding was that males had 30% greater ACL volume and 18% greater ACL width than females. In vivo results of the current study are supported by a previous in vitro study in which males had 35% greater ACL volume and 20% greater cross-sectional area than females

even after adjusting for body height and weight. (Chandrashekar et al., 2005) These results show that in vivo and in vitro sex differences in ACL morphologic measures were similar, with females having consistency smaller ACLs than males.

While lesser ACL volume and ACL width in females was observed, the mechanism(s) by which these findings may directly influence ACL injury risk are not well understood. Theoretically, smaller connective tissue size would be correlated with lower capacity to against external forces.(Nordin & Frankel, 1989) Specific to the ACL, smaller ACL volume has been previously correlated with lower failure load.(Fleming et al., 2011) Further, a computational study stimulated in situ ACL stress, indicating that decreased ACL graft diameter resulted in higher ACL stress.(Westermann, Wolf, & Elkins, 2013) These findings suggest that smaller ACL morphometry could be associated with less restrain capacity thus increase the risk of ACL. Hence, our current study findings of smaller ACL volume and ACL width in females could be a part of an explanation of higher ACL injury rates on females. (Arendt et al., 1999; Arendt & Dick, 1995; Beynnon et al., 2014; Myklebust et al., 1998; Prodromos et al., 2007)

With regard to using an ACL morphometry as a representative of ACL strength and corresponding potential for injury, there does not seem to be a gold-standard method in the literature to fully characterize the ligament's ability to withstand loading. From cadaveric work, both ACL CSA and ACL volume were contributors to ligamentous energy absorbed at failure,(Hashemi, Mansouri, et al., 2011) indicating that multiple dimensional measures such as ACL width, CSA, and volume may represent ligamentous strength.

Given the well-established relationship of CSA to strength, it was surprising that sex differences were not observed for CSA. A potential reason for this may be the methods by which CSA are obtained in vivo. Given the non-uniform 3 dimensional nature of the ACL, CSA and width may not fully represent the entire ACL structure. While ACL width is a single planar measure that does not take into account the non-uniform three dimensional ACL form, the contrast of ligamentous tissue to surrounding tissue is slightly better than measures from the oblique planes used in ACL CSA measures. Additionally the precision of the measures may play a role. Because the ratio of the SEM to the mean differences was 0.19 in ACL width and 7.0 in CSA, the lesser relative precision of CSA measure may have played a role in current findings. Thus with regard to two dimensional measures, ACL width may likely more accurately delineate ACL size than CSA.

Conversely, ACL volume is a three dimensional measure that use multiple sagittal images to fully measure the entire ACL anatomy. This may more fully model the three dimensional nature of the ACL. (Chaudhari et al., 2009; Jamison et al., 2010; Whitney et al., 2014) Further, it has been suggested that three dimensional simulation models using finite element analysis of the ligament could be more predictive of ligamentous biomechanics than one or two dimensional models,(Galbusera et al., 2014) indicating ACL volume may be the best predictor of ligamentous failure characteristics. Collectively, due to the better delineation of ACL morphology, ACL volume may be the best method to represent morphologic on ACL.

The current finding of smaller femoral notch width in females than males (16.4 mm vs. 19.0 mm) is supported by a previous report (15.6 mm vs. 17.7mm). (Uhorchak et al., 2003) Because smaller femoral notch width area has previously been correlated to smaller ACL cross-sectional area, (Dienst et al., 2007) the role that sex differences of ACL morphology have in ACL injury may be influenced by femoral notch width. Therefore, the current study as a secondary objective investigated differences in ACL morphology between males and females after normalizing to the femoral notch width. Current results indicated that the sex differences in ACL size still existed for ACL volume measure regardless of the size of femoral notch width. Thus ACL volume could more precisely represent total ACL morphometry with regard to bony anatomy (Chaudhari et al., 2009; Jamison et al., 2010; Whitney et al., 2014) than ACL width and ACL CSA measures. Additionally, ACL volume is also the only ACL morphometric measure that has been reported as a predictor of ACL injury risk,(Whitney et al., 2014) indicating that ACL volume may be better predictive of ligamentous function and strength. Collectively, ACL volume could be the most appropriate measure to assess morphologic on ACL with regard to studying the sex bias in ACL injury.

Given the limited number of in vivo ACL morphologic studies, the comparison of current values to previously reported values are warranted. The current ACL volume measures compared favorably with previous reports in males (1725.1 mm vs. 1386.0~2256.5 mm^3) and females (1200.1 mm vs. 1072.0~1880.3 mm^3).(Chaudhari et al., 2009; Whitney et al., 2014) Our results of absolute ACL width were similar with a previous in vivo study of males (7.0 m vs. 7.6 m) and females (8.6 m vs. 8.7

m).(Anderson et al., 2001) When comparing our ACL cross-sectional area measures, the current values were higher than previous reported male (0.9 cm^2 vs. 0.5 cm^2) and female (0.8 cm^2 vs. 0.4 cm^2) values.(Anderson et al., 2001) The current difference in ACL cross-sectional area may be based upon the previous discussion of the lack of contrast in our imaging which may be based upon our imaging parameters. Additionally the investigator responsible for the study is not a radiologist, which could likely lend some error to the measure.

The findings from the current study have several clinical implications. From the perspective of structural ACL injury risk factors, our findings provide evidence of in vivo sex differences on various ACL morphometric characteristics. These findings could contribute to future efforts of determining which clinical measures may relate to morphometry in order to better screening targeted populations. In addition, the results provide the options for future imaging studies to determine which ACL size measures could be most suitable regard to investigations of sex bias in ACL injury risk. A better understanding of morphologic factors in ACL injury risk may advance future intervention design.

A limitation of the present study was that all ACL and femoral notch measures required manually segmenting the contour from each MRI image. Depending on the magnetic field strength, chosen sequence, and individual participant variation, there is not a uniform resolution/pixel intensity distinguishing the ACL from surrounding soft tissues. However, our intra-tester consistency of the ACL measures suggests these are reliable measures. A second limitation was that all MRI measures were acquired from the left

knee only. However, this decision was based upon a previous study demonstrating a high degree of ACL volume symmetry ($r = .91$, $P < .001$). (Jamison et al., 2010) A limitation of our secondary objective was that ACL morphometry was normalized by femoral notch width instead of body height and body weight. This decision was based upon previous report of femoral notch width being related to ligamentous cross-sectional area. (Dienst et al., 2007) However, the literature is inconsistent with regard to the relationship of ACL morphology to body size.(Anderson et al., 2001; Charlton et al., 2002; Chaudhari et al., 2009; Fayad et al., 2008; Jamison et al., 2010) Finally, the study was limited by the use of a general healthy population. It is still unknown if the differences found in the current study are found in an only highly athletic population that is at greater risk of sustaining an ACL injury.

Conclusion

In summary, current main findings were that active males had larger ACL volume, ACL width and femoral notch width than active females. Additionally, regardless of the sex differences in femoral notch width, ACL volume of males was still larger than females. Given the previously established association of ACL volume with ACL injury risk (Whitney et al., 2014), ACL volume may be the most relevant measure for investigations of sex difference in ACL injury rate. Future studies are needed to determine the factors associated with smaller ACL morphology in order to better target the high risk injury individuals.

Table 4.1 Descriptive Statistics

	Males		Females	
	Mean± SD	Min-Max	Mean± SD	Min-Max
Age (yrs)	23.2 ± 2.9	19-30	21.3 ± 2.3*	18-27
Height (cm)	180.4 ± 6.7	170-192	166.9 ± 7.7*	151-182
Weight (kg)	84.0 ± 10.9	63-106	61.9 ± 7.2*	51-76
Activating-Rating score	9.2 ± 4.1	4-16	10.7 ± 3.9	4-16
ACL Volume (mm^3)	1712.2 ± 356.3	1052.0-2261.0	1200.1 ± 337.8*	805.2-2231.0
ACL Width (mm)	8.5 ± 2.3	5.3-12.5	7.0 ± 1.2 *	4.7-9.3
ACL CSA (cm^2)	0.9 ± 0.2	0.6-1.3	0.8 ± 0.2	0.4-1.3
Femoral Notch Width (mm)	19.1 ± 1.8	15.9-22.5	16.4 ± 1.1*	13.8-18.8

* statistically different (P <.05) between males and females

Table 4.2 Normalized ACL Morphometric Descriptives

	Males (Mean± SD)	Females (Mean± SD)
Normalized_ACL volume (mm^3/mm)	89.3 ± 15.6	72.4 ± 16.8*
Normalized_ACL Width (mm/mm)	0.44 ± 0.11	0.43 ± 0.07
Normalized_ACL CSA (cm^2/mm)	0.05 ± 0.01	0.05 ± 0.01

* represent statistically different between males and females

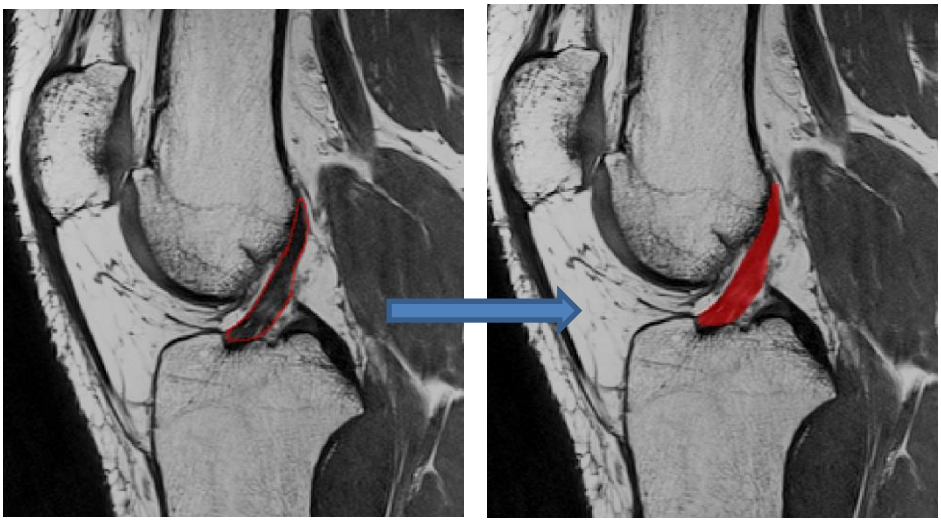

Figure 4.1 ACL Volume Measure. Manual ACL segmentation and resultant area (shaded) on sagittal image.

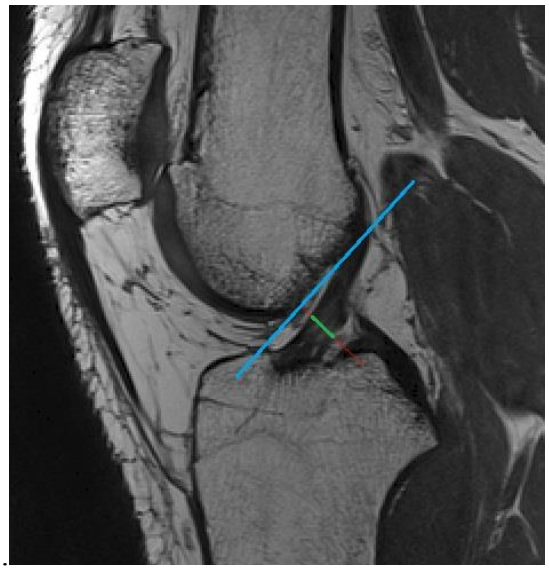

Figure 4.2 ACL Width Measure. The distance across the ACL (green) on a line (red) perpendicular to the Blumensaat's line (blue).

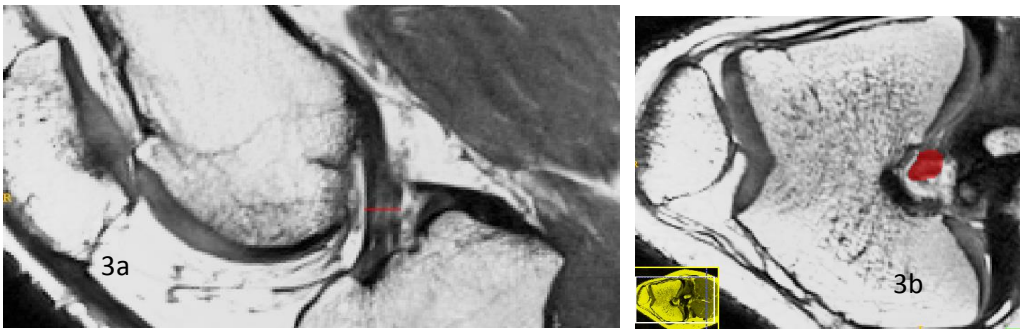

Figure 4.3 CSA Measure. (a) Identified CSA from the oblique sagittal image; (b) The ACL was segmented and CSA calculated from the oblique axial image.

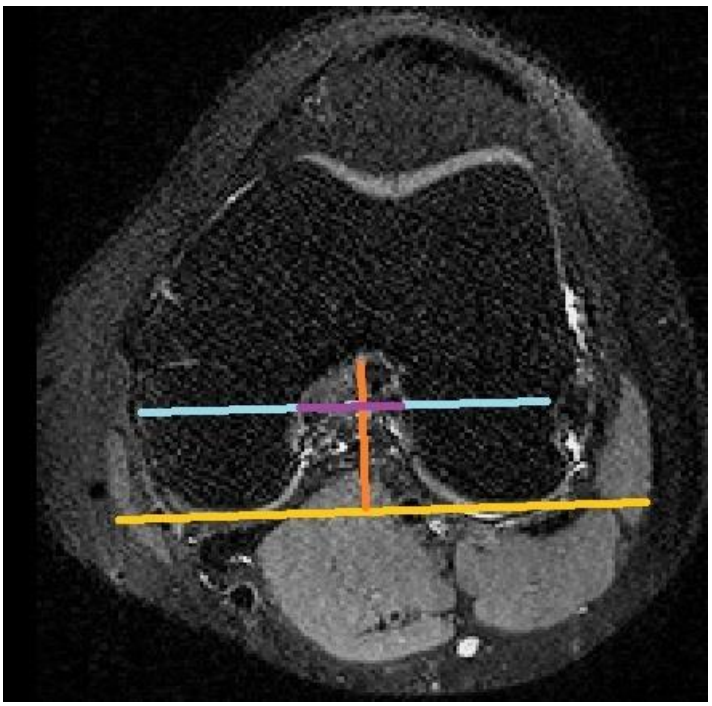

Figure 4.4 Femoral Notch Width Measure. The distance across the notch (purple) taken from a line (blue) parallel to a line tangent to a line located tangent to the posterior femoral condyle located at 2/3 of the notch depth (orange).

CHAPTER V

SEX COMPARISONS OF IN VIVO ANTERIOR CRUCIATE LIGAMENT T2 AND T2* RELAXATION TIME

Abstract

Background: Females have consistently higher ACL injury rates than males. In-vivo sex differences in intrinsic ACL characteristics may offer insight to this injury risk but are not widely understood. Recent advances in quantitative MRI techniques may allow for investigation of sex comparisons of ACL intrinsic properties as assessed via T2 and T2* relaxation times.

Hypothesis: Males have shorter ACL T2 and T2* relaxation times than females.

Study Design: Cross-sectional study

Methods: Recreationally healthy males (n=20) and females (n=20) were assessed via 3T magnetic resonance imaging on the left knee. T2 weighted structural imaging was utilized to calculate ACL volume. T2 relaxation imaging was performed by using spin echo sets with five echo times. T2* relaxation imaging was performed by assessing gradient echo data sets with twelve echo times. Independent sample T-tests examined sex differences in T2 and T2* relaxation times of the ACL.

Results: There were no differences in T2 and T2* ACL relaxation times between sexes.

Conclusion: The current study found no in-vivo differences between active males and females in T2 and T2* relaxation times of human ACLs. Although this indicates that there may be no sex differences in collagen density, collagen ultrastructure organization,

and water content; T2 and T2* relaxation imaging may not be capable of fully characterizing established cadaveric sex differences. Future validation of such imaging techniques is warranted.

Keywords: ACL; MRI; structural composition; knee

Introduction

Intrinsic ligamentous characteristics often refer to both the size and composition of the ligament. With regard to ACL size, ACL volume has been a predictor of ACL injury risk factor (Whitney et al., 2014) and greater ACL graft volume was associated with greater failure load,(Fleming et al., 2011) indicating that ACL morphometry could be indicative of ligamentous strength. Given that females have consistently higher anterior cruciate ligament (ACL) injury rates than males, (Arendt et al., 1999; Arendt & Dick, 1995; Beynnon et al., 2014; Myklebust et al., 1998; Prodromos et al., 2007) the potential role of ACL intrinsic characteristic in this sex bias are not fully understood. (Whitney et al., 2014) While the structural composition of the ligament may also impact ligamentous function and strength,(Culav et al., 1999; Liu et al., 1995; Nakamura et al., 2000; Nordin & Frankel, 1989; Quapp & Weiss, 1997; Raleigh & Collin, 2012) in-vivo sex differences in ACL structural composition are unknown.

The primary compositional components of ligaments include type I collagen, type III collagen, proteoglycans, elastin, and water content.(Culav et al., 1999; Nordin & Frankel, 1989) Lower ACL collagen fibril density in human cadavers has been associated with lower ACL failure strain.(Hashemi, Chandrashekar, Mansouri, et al., 2008) In addition, cadaveric studies have observed that females have lower collagen density, lower

ACL strain at failure, and lower modulus of elasticity when compared to males, (Chandrashekar et al., 2006; Hashemi, Chandrashekar, Mansouri, et al., 2008) indicating compositional sex disparities in ACLs. Collectively, sex differences in ACL structural composition may be associated with ligamentous restraint capacity which may contribute to sex difference in ACL injury risk.

Several structural compositional characteristics of the ligament could impact ligamentous integrity. Regardless of collagen density, the various diameters of different collagen fibers may affect restraint capacity.(Liu et al., 1995) Collagen fibril orientation is also associated with the ability of the ligament to resist external forces.(Quapp & Weiss, 1997) Small amounts of Type V collagen and proteoglycans could determine the structural composition of the larger diameter collagen fibers, which may impact collagen fiber integrity.(Nakamura et al., 2000; Raleigh & Collin, 2012) Such work collectively suggests that for 2 ligaments of the same size, the one that is well-structured and well-organized collagen network matrix may be capable of resisting higher external loads.

To the date we understand very little about in vivo ACL composition. Recent advances in quantitative MRI have allowed insight into material properties of ligamentous tissue. T2 relaxation is referred as the transverse relaxation rate,(Chavhan et al., 2009) with shorter T2 relaxation times in cartilage reflecting denser collagen, more organized collagen ultrastructure, and less water content.(Matzat et al., 2013) Restriction of water molecules in the tissue, thus reducing free water molecules and enhancing dipole-dipole interactions result in shortened T2 relaxation times.(Fullerton & Rahal, 2007; Matzat et al., 2013) Thus, due to the influence of free hydrogen distribution on

MRI signal intensity decay,(Fullerton & Rahal, 2007; Matzat et al., 2013) differences in collagen structure of ligaments may be detected in vivo by T2 relaxation time.(Fleming et al., 2011)

T2 relaxation time has been utilized in an in vivo animal model to predict strength of healing ligamentous graft tissue. Specifically, lower T2 relaxation values when combined with greater ACL volume were associated with greater failure load of animal ACL grafts.(Fleming et al., 2011) Similar to T2 relaxation, T2* relaxation, which considers both the spin-spin interaction and the interaction with the magnetic field, has been utilized in the study of animal ACL grafts.(Biercevicz, Murray, et al., 2014; Chavhan et al., 2009) T2* relaxation time was negatively associated with yield load of healing ACL grafts.(Biercevicz, Murray, et al., 2014) These findings indicate that T2 and T2* relaxation times may both be capable of detecting intrinsic ACL properties.

While T2* relaxation time has been independently associated with ligament strength,(Biercevicz, Murray, et al., 2014) T2 relaxation has not been independently associated with ligament strength.(Fleming et al., 2011) This suggests that T2* relaxation times may be more sensitive in detecting ligament compositional characteristics associated with ligamentous strength than T2 relaxation times. To our knowledge, a direct sex comparison of T2 and T2* relaxation times to assess intrinsic ACL properties has not been performed. Additionally, while T2* relaxation has been utilized in human cadaver ACLs, (Biercevicz, Akelman, Rubin, et al., 2015) the results did not support a previous association with ligamentous graft properties in vivo.(Biercevicz, Murray, et al., 2014) Given the limitation of the cadaver model in fully representing a true physiologic

environment, further human in vivo investigation of T2 and T2* relaxation times with regard to the sex bias in ACL injury is warranted.

A better understanding of detecting potential in-vivo ACL structural compositional differences between sexes could advance future investigations of sex disparities in ACL injury. Thus, the primary objective of the study was to determine sex differences in ACL structural composition as assessed via T2 and T2* relaxation times. It was hypothesized that males have shorter T2* and T2 relaxation times than females.

Materials and Methods

Subjects

Healthy, recreationally active participants (20 males and 20 females) were recruited from local universities to participate in this study (demographics in Table 5.1). Inclusion criteria were: 1) current engagement in sport activities at least 2 hours per week; and 2) no lower extremity injury in the last 6 months. Participants were excluded if they had: 1) previous history of injury to the capsule, ligament, or menisci of either knee 2) any vestibular or balance disorder and 3) any metal or implanted medical device in the body. All participants read and sign an informed consent form approved by the University of North Carolina at Greensboro's Institutional Review Board for the Protection of Human Subjects. Each participant attended a MRI testing session consisting of structural T2 imaging along with T2 and T2* relaxation mapping imaging. Participants were instructed to avoid high intensity activities 24 hours prior to testing. All measures were performed on the left knee. In order to control potential hormonal effects on collagen metabolism, females were tested during a limited window of the menstrual cycle (3 to 8

days post menses onset). (Shultz, Wideman, et al., 2012) The activity rating scale (Marx et al., 2001) was used to quantify participant activity level. Participants self-rated running, cutting, decelerating, and pivoting activities each as 0 (less than once per month), 1 (once per month), 2 (once per week), 3 (2–3 times per week) or 4 (4 or more times per week), resulting in a score from 0 to 16.

MRI Examination

All MRI data were acquired using 3T Siemens Tim Trio scanner (Erlangen, Germany) and a 15 channel knee coil (Siemens Erlangen, Germany). T2-weighted, multiplanar MRI scans (repetition time (TR) =1300 ms; excitation time (TE) = 39 ms; Flip angle (FA) =160º; FOV=150x150 mm; voxel size= 0.5×0.5×0.5mm) were used to calculate ACL volume.

The T2 relaxation imaging was performed using spin echo data sets with following parameters: repetition time (TR) =3040 ms; excitation time (TE) at 13.8, 27.6, 41.4, 55.2, and 69 ms; flip angle (FA) =180º; voxel size, FOV=160 x160 mm; voxel size= 0.4×0.4×3.0mm.(Fleming et al., 2011) T2* relaxation was performed using gradient echo data sets with following parameters: repetition time (TR) =1000 ms; excitation time (TE) at 8.26, 10.28, 12.3, 14.32, 16.34, 18.36, 20.38, 22.4, 24.42, 26.44, 28.46 and 30.48 ms; flip angle (FA) =90º; FOV=280 x280mm; voxel size = 0.5×0.5×3.0mm.(Biercevicz, Akelman, et al., 2014; Biercevicz, Akelman, Rubin, et al., 2015)

Morphometric Data Reduction

ACL volume was calculated as reported by Chaudhari et al (Chaudhari et al., 2009) using ITK-SNAP software (http://www.itksnap.org/pmwiki/pmwiki.php). ACL

contouring of each sagittal slice was done manually using a digitizing tablet (Wacom DTK1300; Wacom Co, Kazo, Japan). All slices that were contoured were used in the calculation of ACL volume and then in the creation of 3D ACL volume (Figure 5.1). For the establishment of intra-tester reliability and precision, ACL volume in 10 pilot participants was measured twice at least a week apart [$ICC_{3,1}$ (SEM) =0.97 (36.1) mm^3].

MRI Relaxation Time Data Reduction

Using customized Matlab code (Mathworks Inc, U.S.A), the voxel-wise T2 relaxation maps were calculated using the signal intensity (SI) relationship from all five echo times of the T2 relaxation imaging sequence. Equation: SI (TE) = So exp(-TE/T2), where SI(TE) are the voxel-specific SIs for the various echo times (TE) and where So is the signal intensity at the initial TE.(Fleming et al., 2011) A graphic representation of the calculation of relaxation maps is located in Figure 5.2. To isolate ligament specific T2 values, the calculation T2 relaxation map was registered to the structural imaging sequence using Slicer 3D Software (https://www.slicer.org/) and the mean T2 relaxation value of the voxels (Figure 5.3) included in the 3D ACL volume described above was calculated using ITK SNAP software (http://www.itksnap.org/pmwiki/pmwiki.php) and included in the analyses.

Using customized Matlab code (Mathworks Inc, U.S.A), the voxel-wise T2* relaxation maps were calculated using the signal intensity (SI) relationship from all twelve echo times of the T2* relaxation imaging sequence. Equation: SI (TE) = So exp(-TE/T2*), where SI(TE) are the voxel-specific SIs for the various echo times (TE) and where So is the signal intensity at the initial TE.(Biercevicz, Akelman, et al., 2014;

Biercevicz, Akelman, Rubin, et al., 2015) To isolate ligament specific T2* values, the calculation T2* relaxation map was registered to the structural imaging sequence using Slicer 3D Software (https://www.slicer.org/) and the mean T2* relaxation value of the voxels included in the 3D ACL volume described above was calculated using ITK SNAP software (http://www.itksnap.org/pmwiki/pmwiki.php) and included in the analyses.

Statistical Analysis

Independent sample T-tests examined sex differences in T2 and T2* ACL relaxation times. The alpha level for all analyses was set priori at equal or less than .05. All calculations were performed using the SPSS statistical software. (version 21.0; IBM Corp, Armonk, NY)

Results

Descriptive statistics for the mean ACL T2 relaxation and T2* relaxation times between males and females are show in Table 5.1. There were no significant differences in T2 relaxation time ($T_{.05\ (1,\ 38)} = -.61$, p=.543), and T2* relaxation times ($T_{.05\ (1,\ 38)} = -.83$, p=.412) between males and females.

Discussion

Structural composition of cadaver ACLs has been associated with failure load (Chandrashekar et al., 2006; Hashemi, Chandrashekar, Mansouri, et al., 2008) which indicates that intrinsic ACL properties may play a role in ACL injury. However, the influences of potential in-vivo sex disparities in ACL structural composition that may contribute to the sex bias in ACL injury rates are unknown. In the present study, we compared the differences between males' and females' ACL structural composition as

assessed via T2 and T2* relaxation mapping. Our main result demonstrated that males had similar T2 and T2* relaxation times compared to females, which suggested no structural composition differences of the ACL.

We are unaware of other in vivo sex comparisons of ligamentous tissue. Thus, sex comparisons in other similar tissue may help to interpret current findings. Knee articular cartilage T2 relaxation times of healthy males (57.9 ± 5.2 ms) was similar to healthy females (57.0 ± 5.3 ms),(Mosher et al., 2004) which is congruent with the current T2 findings of in vivo ACL T2 relaxation times of males (58.2 ± 7.7 ms) and females (55.9 ± 8.1 ms). While studies establishing validation of T2 and T2* relaxation times to tissue properties have focused on articular cartilage tissue,(H. K. Kim, Shiraj, Anton, Horn, & Dardzinski, 2014; Mosher et al., 2004; Watrin et al., 2001) the current study was the first in vivo report of ACL T2 and T2* relaxation times. When comparing the structure of articular cartilage to ligamentous tissue, both tissues have an abundance water and an extracellular matrix.(Bhosale & Richardson, 2008; C. Frank et al., 1985; C. B. Frank, 2004; Sophia Fox, Bedi, & Rodeo, 2009) While 65-80% of cartilage is composed of water, type II collagen accounts for 10-20% of wet weight and proteoglycans account for 10-20% of the wet weight.(Bhosale & Richardson, 2008; Sophia Fox et al., 2009) Conversely, slightly less of the ligament is composed of water (~67% of wet weight) with type I and type III collagen accounting for ~25% of the wet weight and the other being small amounts of elastic, proteoglycans and glycoproteins.(C. B. Frank, 2004; C. B. Frank et al., 1999) While there are structurally similarities between articular cartilage and ligament that warrant investigation of T2 and T2* relaxation

imaging in ligament, the structural differences between the tissues have the potential to override the established sensitivity of T2 and T2* relaxation times to water and collagen content in cartilage.(Li et al., 2011; Mosher et al., 2000; Regatte et al., 2002; Wayne et al., 2003; White et al., 2006)

Comparison of T2 and T2* relaxation times between the cartilage and ligaments help us to further understand the sensitivity of T2 and T2* measures in various tissues. In knee cartilage measures, T2 relaxation times have been reported around 58.3 ±14.4 ms in vivo(Welsch et al., 2008) and 51.9 ± 9.2 ms in vitro (T. Kim et al., 2014) while T2* relaxation times were around 22.5 ± 7.7 ms(Welsch et al., 2008) in vivo and 20.3 ± 10.3 ms(T. Kim et al., 2014) in vitro. The present study reported that mean T2 relaxation was 57.1 ± 7.9 ms and mean T2* relaxation was 18.9 ± 2.4 ms. While mean values are similar between cartilage and ligamentous tissue, we are still unaware of work that has fully investigated the relationship T2 and T2* measures to ligamentous tissue components. Hence, future investigations of histologic studies in healthy ligaments are needed.

Although a previous cadaver study reported 18% less ACL collagen density in females in than males,(Hashemi, Chandrashekar, Mansouri, et al., 2008) current findings did not support in vivo sex differences in ligamentous structure composition as assessed by T2 and T2* relaxation times. Similarly, T2* relaxation could not predict maximum failure in combined sex cadaveric ACLs.(Biercevicz, Akelman, Rubin, et al., 2015) Based on the association of ligamentous structural composition to failure load, (Chandrashekar et al., 2006; Hashemi, Chandrashekar, Mansouri, et al., 2008) these previous findings suggest that T2* relaxation may not have the sensitivity to detect collagen density

differences previously assessed in cadavers by transmission electron microscopy.(Hashemi, Chandrashekar, Mansouri, et al., 2008) However, sex comparisons of other intrinsic characteristics such as collagen orientation and proteoglycan content which may affect the network matrix and corresponding T2 and T2* relaxation times are unknown.

Regardless of sex comparisons, several animal studies have reported that shorter T2 and T2* relaxation times were associated with larger ligamentous graft failure load,(Biercevicz, Murray, et al., 2014),(Fleming et al., 2011) indicating that structural composition could be associated with ligament strength. However, in vivo T2 and T2* relaxation times have not been studied in healthy ligamentous tissue. A single previous cadaveric ACL study reported a T2* range from 10.6-17.7ms (Biercevicz, Akelman, Rubin, et al., 2015) and median was 13.1 ms compared to the current study T2* range was of 13.0-24.6 ms and median was 18.7. Although relaxation times may be slightly higher in vivo than in vitro, the in vivo and in vitro abilities of T2 and T2* relaxation times to characterize ligamentous ultrastructure characteristics in healthy ligaments is needed.

The present study was limited by lack of direct measurement of ligamentous histology. It is important to note that the previous T2 and T2* findings are related to the collagen characteristics and water content as studied in articular cartilage.(Li et al., 2011; Mosher et al., 2000; Regatte et al., 2002; Wayne et al., 2003; White et al., 2006) With regard to ligamentous tissue, existing work is limited to the T2 and T2* relaxation times relationships to ligamentous biomechanics.(Biercevicz, Akelman, Rubin, et al., 2015;

Biercevicz, Murray, et al., 2014; Fleming et al., 2011) A second limitation is that potential factors affecting structural composition of the ACL are not fully understood. Our current work did not account for the multitude of genetic factors that could impact structural composition of the ACL.(Raleigh & Collin, 2012) However, how mechanism(s) by which genetic profiles alter ligamentous structural composition and corresponding ligamentous function and strength are inconclusive. Finally, although activity-rating scores were similar between sexes (Table 1), participants had a wide range of physical activity levels which may impact ligamentous structural composition.(Tipton et al., 1975)

Conclusion

In summary, the current study found no in-vivo differences between active males and females in T2 and T2* relaxation times in human ACLs. Although this indicates that there may be no sex differences in collagen density, collagen ultrastructure organization, and water content; T2 and T2* relaxation imaging may be not sufficient to fully characterize established cadaveric sex differences. While sex differences in T2 and T2* relaxation times were not identified, the current study offers a reference for future comparisons with pathologic or high-risk individuals. Future histologic studies are needed to determine the extent of which structural composition of ligament is sensitive to T2 and T2* relaxation times.

Table 5.1 Descriptive Statistics

	Male (N=20)		Female (N=20)	
	Mean ± SD	Min-Max	Mean ± SD	Min-Max
Age (yrs)	23.2 ± 2.9	19-30	21.3 ± 2.3*	18-27
Height (cm)	180.4 ± 6.7	170-192	166.9 ± 7.7*	151-182
Weight (kg)	84.0 ± 10.9	63-106	61.9 ± 7.2*	51-76
Activity-Rating score	9.2 ± 4.1	4-16	10.7 ± 3.9	4-16
T2 Relaxation (ms)	58.2 ± 7.7	42.1-67.4	55.9 ± 8.1	42.7-69.5
T2* Relaxation (ms)	19.4 ± 2.6	14.5-24.6	18.5 ± 2.2	13.0-22.6

* represent statistically different ($P < .05$) between males and females

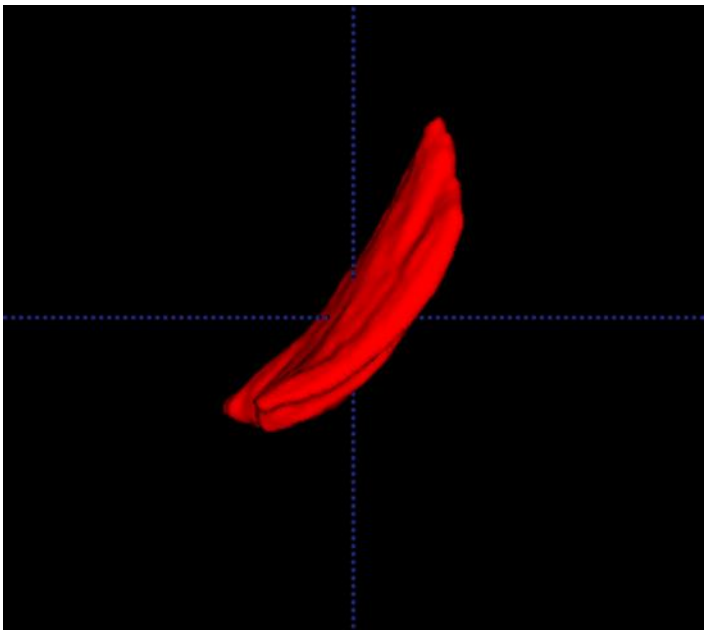

Figure 5.1 3D ACL Model. 3D ACL model ascertained from segmented images.

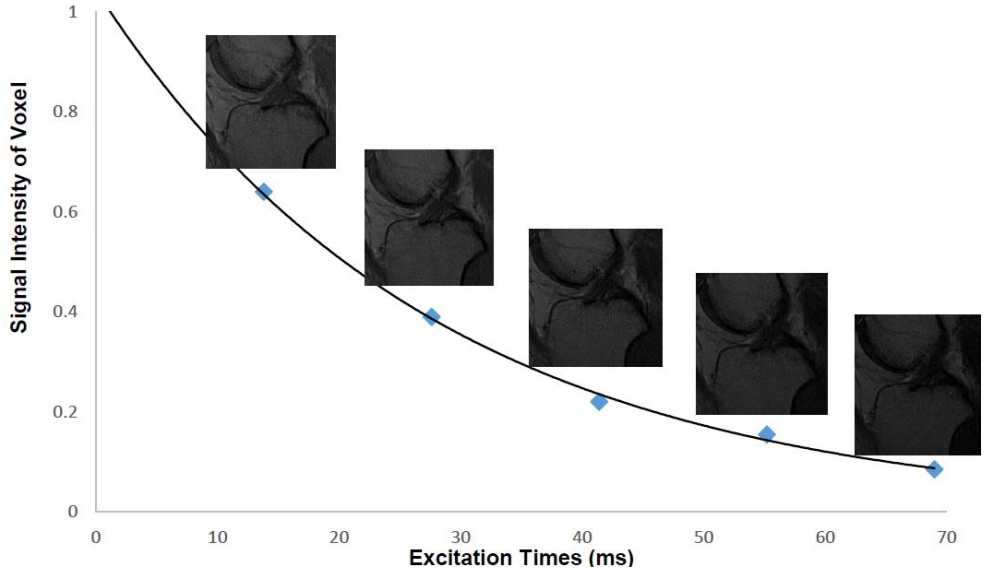

Figure 5.2 Example of T2 Relaxation Time. Example of T2 relaxation time quantification using the voxel-wise data from each of the 5 excitation times. The resultant rate of voxel by voxel signal intensity decay was quantified over the 5 times using the Levenberg-Marquardt monoexpoential equation (Fleming et al., 2011)

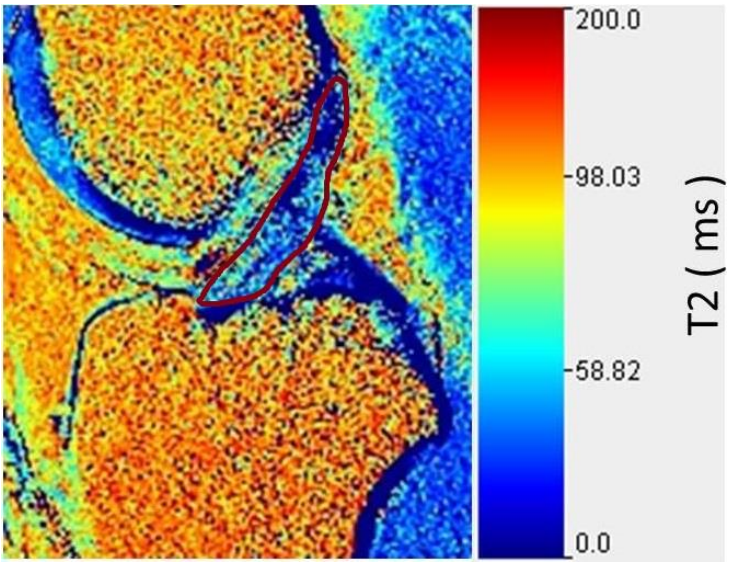

Figure 5.3 Example of T2 Relaxation Map. Example of T2 relaxation map was ACL outlined by brown contour.

CHAPTER VI

THE RELATIONSHIP OF ACL MORPHOMETRY AND STRUCTURAL COMPOSITION TO ANTERIOR KNEE LAXITY

Abstract

Background: Greater anterior knee laxity (AKL) has been reported as a prospective ACL injury risk factor and could be indicative of a structurally weaker ligament. Given that ACL morphometry and structural composition have the potential to influence ligamentous strength, thus its biomechanical response to an applied load, understanding the combined contributions of ACL morphometry and structural composition is warranted.

Hypothesis: ACL volume and T2* relaxation times are the strongest morphometric and relaxation time predictors, respectively, of AKL in both sexes. Smaller ACL volume combined with lower T2* relaxation times would collectively predict greater AKL in both sexes.

Study Design: Cross-sectional study

Methods: College-aged, active healthy males (n=20) and females (n=20) underwent MRI examination and AKL testing on the left knee. T2 weighted MRI scans assessed ACL volume, ACL width, and ACL cross-sectional area. T1 weighted MRI scans assessed femoral notch width. T2 and T2*relaxation times assessed ACL structural composition. AKL was measured via a commercial knee arthrometer. After determining the strongest independent morphometric and relaxation times prediction measures of AKL, separate

sex-specific linear regressions examined the degree to which ligament morphometry and MR relaxation time collectively predicted AKL.

Results: T2 relaxation time was an independent predictor of AKL in females and ACL volume was the strongest ACL size predictor in both males and females. In the multivariate model smaller ACL volume and lower T2 relaxation times collectively predicted greater AKL in females, while smaller ACL volume and larger T2* relaxation times predicted greater AKL in males.

Conclusion: Smaller ACL volume combined with either lower T2 in females or higher T2* relaxation times in males predict greater AKL. The findings that ACL morphometry and structural composition features individually and collectively contribute to AKL lay a basis for in vivo investigations focused on AKL as a risk factor of non-contact ACL injury.

Keywords: ACL volume; MRI; T2 relaxation times; Knee

Introduction

Anterior cruciate ligament (ACL) injury frequently occurs in active populations with around 70% of ACL injuries resulting from non-contact injury mechanisms.(Boden et al., 2000; Gianotti et al., 2009; Hootman et al., 2007) The primary function of the ACL is to prevent anterior displacement of the tibia relative to the femur(Butler et al., 1978) with secondary functions to protect against increased knee abduction and tibial rotation motions.(Markolf et al., 1995) While knee stability during functional activity is provided both passively by the ligaments and actively by the muscles around the knee,(Noyes et al., 1980) it is possible that when there is a delay or an error in the neuromuscular control

system, active restraint is insufficient and a greater relative demand is placed on the passive restraints.(Hashemi, Breighner, et al., 2011; Hewett et al., 2002) During this situation, the capability of the ligament to resist the external load is critical in maintaining ACL integrity.

Clinically, ACL function is most commonly assessed by anterior knee laxity (AKL) testing.(Butler et al., 1980) AKL is commonly defined as the anterior displacement of the tibia relative to the femur under a fixed load with greater AKL being prospectively identified as an ACL injury risk factor.(Branch et al., 2010; Myer et al., 2008; Uhorchak et al., 2003; Woodford-Rogers et al., 1994) The mechanisms underpinning this increased risk of injury with greater AKL are not well understood. Greater anterior-posterior knee laxity was associated with lower failure load one year after ACL reconstruction surgery in a canine population.(Beynnon et al., 1994) Additionally, 3D finite element modeling reported that greater PCL graft laxity was associated with lower graft strength.(Lai et al., 2015) While these studies are limited to ligamentous grafts, they suggest that greater knee laxity could be indicative of a weaker ligament. Thus, factors associated with lesser laxity have the potential to be related to ligamentous strength. A better understanding of the factors associated with stronger or less lax ligaments may be of benefit in ultimately reducing ACL injury incidence.

The orthopedic biomechanics literature has well established that greater material size is positively related to the ability to resist external loadings, thus producing less displacement of the tissue when loaded.(Nordin & Frankel, 1989) Focusing on the ACL, this theory would indicate that greater ACL morphometry (i.e. larger size) would be

associated with less deformation of the ligament under the fixed anterior load, thus less AKL. While this theory is supported by total anterior-posterior translation of animal knees being associated with cross-sectional area of the reconstructed ACL (R^2=.86), (Grood et al., 1992) the relationship between ACL morphometry as measured by ligament width and AKL in healthy humans (R^2=.22) was relatively weak compared to the previous animal study.(H.-M. Wang et al., 2015) Such differences in laxity's relationships with ligament size are likely explained in part by the different morphometric measures as the most ACL size predictive of ligament function has yet to be established.

The restraint capacity of the ligament may not be fully represented by the ligamentous morphometric characteristics. Intrinsic factors such as of collagen fiber orientation,(Woo et al., 1991) collagen density,(Amiel et al., 1989) and collagen fiber diameter (Culav et al., 1999; Liu et al., 1995) also have the potential to impact ligamentous function. These intrinsic factors could help to stabilize the extracellular matrix thus increasing resistance to deformation under load. Thus, intrinsic factors contributing to stronger/less lax ligaments should also be investigated in vivo.

While there is no current gold-standard to detect in vivo ligamentous structural composition, quantitative magnetic resonance imaging (MRI) has the potential to offer compositional insight. T2 relaxation times are related to cartilage collagen density, collagen orientation, and water content.(Matzat et al., 2013) This suggests structural composition of ligaments may also be measured via MRI. Lower T2 relaxation times were correlated with larger failure load and lower anterior-posterior knee laxity in animal ACL grafts.(Fleming et al., 2011) Additionally, lower T2* relaxation times were related

to greater yield load on healing ACL grafts.(Biercevicz, Murray, et al., 2014) Thus, T2 and T2* relaxation times may both be utilized to detect ligamentous structural composition and could be associated with AKL. A better understanding of which quantitative MRI measure best predicts AKL and associated structural composition would further advance our understanding of ACL injury.

While greater AKL is an established ACL injury risk factor,(Branch et al., 2010; Myer et al., 2008; Uhorchak et al., 2003; Woodford-Rogers et al., 1994) there is much to be understood as to the factors that contribute to greater AKL. Thus, the first purpose was to determine which MR relaxation measure and which ACL morphometric measure were the strongest independent predictors of AKL. It was hypothesized that T2* relaxation times and ACL volume would have the greatest independent predictive abilities of AKL. Next, the primary objective of the study was to determine the extent to which ACL structural composition and ACL morphometry combine to predict to AKL in active females and males. It was hypothesized that smaller ACL morphometry and longer T2* relaxation times would combine to predict greater AKL in males and females than morphometry or relaxation time in isolation. A better understanding of the relationship of the intrinsic ligamentous factors to AKL may serve to inform future prevention programming to address the established risk factor of greater AKL by focusing on increasing ligamentous strength.

Materials and Methods

Subjects

Healthy, recreationally active participants (20 males and 20 females) were recruited from local universities to participate in this study (demographics in Table 6.1). Inclusion criteria were: 1) current engagement in sport activities at least 2 hours per week; and 2) no lower extremity injury in the last 6 months. Participants were excluded if they had: 1) previous history of injury to the capsule; ligament, or menisci of either knee; 2) any vestibular or balance disorder; 3) any metal or implanted medical device in the body; 4) did not meet predefined AKL criteria(Shultz et al., 2007); or 5) could not relax during the AKL measures. All participants read and sign an informed consent form approved by the University of North Carolina at Greensboro's Institutional Review Board for the Protection of Human Subjects. Each participant attended an AKL testing session as well as a MRI testing session consisting of structural T1 and T2 imaging along with T2 and T2* relaxation mapping imaging. Participants were instructed to avoid high intensity activities 24 hours prior to testing. All measures were performed on the left knee. In order to control potential hormonal effects on AKL, females were tested during a limited window of the menstrual cycle (3 to 8 days post menses onset). (Shultz et al., 2004) The activity rating scale (Marx et al., 2001) was used to quantify participant activity level. Participants self-rated running, cutting, decelerating, and pivoting activities each as 0 (less than once per month), 1 (once per month), 2 (once per week), 3 (2–3 times per week) or 4 (4 or more times per week), resulting in a score from 0 to 16.

Anterior Knee Laxity Assessment

AKL was defined as the anterior displacement of the tibia relative to the femur at 130 N load and was measured by a single examiner using the KT-2000 Knee Arthrometer (Medmetric Corp, San Diego, CA). The subject was placed in a supine position with the knee flexed 25 ± 5° over a thigh bolster. The foot/ankle was rested in the foot cradle while a hook and loop strap was placed around both thighs. This method is used to prevent rotation of the lower extremities during testing. The examiner was first to apply 90 N posterior-directed force then 130 N anterior-directed force to the tibia, while displacement (mm) of the tibia with respect to the femur is recorded by computer software. Three measures were obtained and the last two averaged for analyses. AKL was defined as the average anterior displacement of the tibia relative to the femur over the last two trials. The investigator has previously established between day measurement consistency and precision [ICC (SEM) =0.87 (0.5) mm] of this measure.(Taylor et al., 2015) Potential subjects were prescreened to obtain a wide distribution of AKL values in both sexes. This was done to ensure equal amounts of average, above-average, and below average laxity within each sex. Previously reported data (Shultz et al., 2007) was used to define average (M=5.6 ± 1.0 mm, F=8.1 ± 2.5 mm) above-average (>1 SD; M=6.6mm, F=10.6mm), and below average (<1 SD; (M=4.6mm, F=5.6mm) AKL.

MRI Examination

MRI data were acquired using a 3T Siemens Tim Trio scanner (Erlangen, Germany) and a 15 channel knee coil (Siemens Erlangen, Germany). T2-weighted, multiplanar MRI scans (repetition time (TR) =1300 ms; excitation time (TE) = 39 ms;

Flip angle (FA) =160º; FOV=150x150 mm; voxel size = 0.5×0.5×0.5mm) were used for ACL morphometric measures. T1-weighted, multiplanar MRI scans (repetition time (TR) =1200 ms; excitation time (TE) = 33 ms; FOV=160x160 mm; voxel size = 0.5×0.5×0.6mm) were used for femoral notch width measures.

The T2 relaxation imaging was performed using spin echo data sets with following parameters: repetition time (TR) =3040 ms; excitation time (TE) at 13.8, 27.6, 41.4, 55.2, and 69 ms; flip angle (FA) =180º; voxel size, FOV=160 x160 mm; voxel size= 0.4×0.4×3.0mm.(Fleming et al., 2011) T2* relaxation was performed using gradient echo data sets with following parameters: repetition time (TR) =1000 ms; excitation time (TE) at 8.26, 10.28, 12.3, 14.32, 16.34, 18.36, 20.38, 22.4, 24.42, 26.44, 28.46 and 30.48 ms; flip angle (FA) =90º; FOV=280 x280mm; voxel size = 0.5×0.5×3.0mm.(Biercevicz, Akelman, et al., 2014; Biercevicz, Akelman, Rubin, et al., 2015)

MRI Morphometric Data Reduction

ACL volume was calculated as reported by Chaudhari et al (Chaudhari et al., 2009) using ITK-SNAP software (http://www.itksnap.org/pmwiki/pmwiki.php). ACL contouring of each sagittal slice was done manually using a digitizing tablet (Wacom DTK1300; Wacom Co, Kazo, Japan). All slices that were contoured were used in the calculation of ACL volume (Figure 6.1). For the establishment of intra-tester reliability and precision, ACL volume in 10 pilot participants was measured twice at least a week apart [$ICC_{3,1}$ (SEM) =0.97 (36.1) mm^3].

ACL width was measured using Medical Image Processing, Analysis and Visualization software (MIPAV; http://mipav.cit.nih.gov) per methods described

previously.(Anderson et al., 2001) First, the sagittal plane image that indicated the clearest image of Blumensaat's line was selected. Blumensaat's line is the landmark which corresponds to the roof of femoral intercondylar notch as drawn on the sagittal knee joint image.(Seyahi et al., 2006) At the point of the notch outlet, ACL width was determined by a line drawn perpendicular to Blumensaat's line that measured the distance across the ACL (Figure 6.2). The investigator has previously established intratester measurement consistency and precision [$ICC_{3,1}$ (SEM) =0.98 (0.3) mm] of this measure.(H.-M. Wang et al., 2015)

ACL cross-sectional area was measured using ITK-SNAP software (http://www.itksnap.org/pmwiki/pmwiki.php) as reported by Whitney et al.(Whitney et al., 2014) From an oblique sagittal image perpendicular to the ACL, a point one third of the total ACL length from the attachment to the tibia was identified (Figure 6.3a). After identification of this point, ACL cross-sectional area was segmented and calculated from the oblique axial image (Figure 6.3b). For the establishment of intra-tester reliability and precision, ACL cross sectional area in 10 pilot participants was measured twice at least a week apart [$ICC_{3,1}$ (SEM) =0.87 (0.7) cm^2].

Femoral notch width was measured using Medical Image Processing, Analysis and Visualization software (MIPAV; http://mipav.cit.nih.gov) as reported by Stein et al.(Stein et al., 2010) First, the clearest image of Blumensaat's line from the sagittal image and the beginning of the Blumensaat's line at the anterior outlet from the frontal image were chosen to identify the axial image. Then, from the axial image, the articular surface line tangent to the medial and the lateral femur condyle was drawn. Notch depth

is the line perpendicular to the articular surface line. At two-thirds of the notch depth, the notch width was calculated as the line parallel to the articular surface line (Figure 6.4). For the establishment of intra-tester reliability and precision, femoral notch width in 10 pilot participants was measured twice at least a week apart [$ICC_{3,1}$ (SEM) =0.99 (0.2) mm].

MRI Structural Composition Data Reduction

Using customized Matlab code (Mathworks Inc, U.S.A), the voxel-wise T2 relaxation maps were calculated using the signal intensity (SI) relationship from all five echo times of the T2 relaxation imaging sequence. Equation: SI (TE) = So exp(-TE/T2), where SI(TE) are the voxel-specific SIs for the various echo times (TE) and where So is the signal intensity at the initial TE.(Fleming et al., 2011) A graphic representation of the calculation of relaxation maps is located in Figure 6.5. To isolate ligament specific T2 values, the calculated T2 relaxation map was registered to the structural imaging sequence using Slicer 3D Software (https://www.slicer.org/) and the mean T2 relaxation value of the voxels (Figure 6.6) included in the 3D ACL volume (Figure 6.7) described above was calculated using ITK SNAP software (http://www.itksnap.org/pmwiki/pmwiki.php) and included in the analyses.

Using customized Matlab code (Mathworks Inc, U.S.A), the voxel-wise T2* relaxation maps were calculated using the signal intensity (SI) relationship from all twelve echo times of the T2* relaxation imaging sequence. Equation: SI (TE) = So exp(-TE/T2*), where SI(TE) are the voxel-specific SIs for the various echo times (TE) and where So is the signal intensity at the initial TE.(Biercevicz, Akelman, et al., 2014;

Biercevicz, Akelman, Rubin, et al., 2015) To isolate ligament specific T2* values, the calculation T2* relaxation map was registered to the structural imaging sequence using Slicer 3D Software (https://www.slicer.org/) and the mean T2* relaxation value of the voxels included in the 3D ACL volume described above was calculated using ITK SNAP software (http://www.itksnap.org/pmwiki/pmwiki.php) and included in the analyses.

Statistical Analysis

Separate sex-specific stepwise linear regression analyses tested the initial hypotheses that T2* relaxation times and ACL volume would have the strongest independent predictive ability of AKL for quantitative MR imaging and ACL morphometry, respectively. When assessing the relationship of ACL morphometry and AKL, femoral notch width was initially entered to control for the relationship of ACL size and femoral notch width. (Dienst et al., 2007) On the next step of the ACL morphometry regression, the stepwise forward method was used to assess which ACL morphometry variable was the strongest AKL predictor in males and females. The independent variables were ACL volume, ACL width and ACL CSA. For the quantitative MR imaging regression, forward stepwise method was used to assess which quantitative MR imaging variable was the strongest AKL predictor in males and females. The independent variables were T2 relaxation and T2* relaxation times with AKL serving as the dependent variable.

Separate sex-specific stepwise linear regression analyses tested the primary hypothesis that the ACL MR relaxation time and ACL morphometric measure, as determined from the initial hypotheses, would each have unique contributions in a

multivariate model explaining the variance in AKL. The independent variables were the ACL MR relaxation time and ACL morphologic measure as determined from the initial hypotheses with the dependent variable being AKL. In the regression model, stepwise linear regression analysis was used to assess the combined predictive ability of the included morphometric and relaxation time variables. The alpha level for all analyses was set priori at equal or less than .05. All calculations were performed using the SPSS statistical software (version 21.0; IBM Corp, Armonk, NY)

Results

Descriptive statistics for AKL, ACL volume, ACL width, ACL cross-sectional area, femoral notch width, T2 relaxation times and T2* relaxation times are shown in Table 6.1. Obtained laxity distributions are found in Table 6.2. A pearson correlation table of all variables can be found in Table 6.3. On the initial step of the sex-specific stepwise ACL morphometry regression analyses, femoral notch width was significantly associated with AKL in females ($R^2 = .22$, $P = .039$) and males ($R^2 = .21$, $P = .041$) with ACL volume in females being the only ACL morphometric factor to explain additional variance ($R^2\Delta=25\%$, $P\Delta=.012$). Although not explaining significant additional variance in males, ACL volume was the first ACL morphometric measure to enter ($R^2\Delta=9\%$, $P\Delta=.149$). Both ACL width and ACL CSA were excluded from the stepwise regression model. These sex-specific stepwise regression models and regression coefficients are found in Tables 6.4 and 6.5, respectively. From these findings, ACL volume was chosen as the ACL size measure to be included in the subsequent regressions to test the relationship of ACL size and relaxation times to AKL. The final regression equation in

females was AKL= (-.074)*femoral notch width + (-.005)*ACL volume + 15.631; the final regression equation in males was AKL= (-.485)*femoral notch width + 15.581.

On the initial step of the sex-specific stepwise MR relaxation times regression analyses, T2 relaxation time in females was significantly associated with AKL (R^2 = .27, P = .020) and T2* relaxation times could not explain additional variance ($R^2\Delta$=6%, PΔ=.239). In males, T2* relaxation time was a nonsignificant trend to predict AKL (R^2 = .15, P = .098) and T2 relaxation times could not explain additional variance ($R^2\Delta$=6%, PΔ=.287). These sex-specific stepwise regression models and regression coefficients are found in Tables 6.6 and 6.7, respectively. From these findings, T2 and T2* were chosen for females and males, respectively, as the MR relaxation times to be included in the subsequent regressions to test the relationship of ACL size and relaxation times to AKL. The final regression equation in females was AKL= (-.171)*T2 relaxation times + 17.634.

The final sex-specific stepwise linear regression analyses were used to test the ability of ACL volume and T2 (females) or T2* (males) relaxation times to collectively predict AKL. On the initial step of the sex-specific regression analyses ACL volume was significantly associated with AKL in both females (R^2 = .47, P = .001) and males (R^2 = .28, P = .017). In females, smaller ACL volume combined with lower T2 relaxation times significantly predicted greater AKL (R^2 = .68, P< .001; $R^2\Delta$=22%, PΔ=.003); whereas, smaller ACL volume combined with higher T2* relaxation times significantly predicted greater AKL in males (R^2 = .44, P = .008; $R^2\Delta$=16%, PΔ=0.043). The sex-specific stepwise regression models and regression coefficients are found in Tables 6.8

and 6.9, respectively. The final regression equation in females was AKL= (-.005)*ACL volume + (-.155)*T2 relaxation times + 22.963; the final regression equation in males was AKL= (-.003)*ACL volume + (.310)*T2* relaxation times + 5.381.

Discussion

Greater AKL has been identified as a prospective risk factor of non-contact ACL injury. (Branch et al., 2010; Myer et al., 2008; Uhorchak et al., 2003; Woodford-Rogers et al., 1994) However, little is understood how ACL size and structural composition characteristics may contribute to a more lax knee. As hypothesized, our primary findings in males were that smaller ACL volume combined with higher ACL T2* relaxation time predicted greater AKL. Contrary to our hypothesis, smaller ACL volume combined with lower ACL T2 relaxation time predicted greater AKL in females. To the best of our knowledge, the current study was the first in vivo report of the relationship of AKL to ACL volume and quantitative tissue relaxation times; indicating that ACL size and ACL structural composition independently contribute to AKL.

While the final overall regression models predicting AKL from ACL size and relaxation times were significant in females as well as males (Table 8), examination of the regression coefficients indicates that ACL volume and the respective relaxation times were each significant independent predictors of AKL (Table 9). It is of note in the final female model that the partial correlation of ACL volume trended upward from -.683 (P = .001) to -.755 (P<.001). Similarly in males the partial correlation of ACL volume trended upward from -.528 (P = .017) to -.584 (P =.043). These findings together indicate

that ACL volume and relaxation times can independently and collectively contribute to AKL.

While previous animal and human studies reported that smaller ACL size was associated with greater AKL, (Grood et al., 1992; H.-M. Wang et al., 2015) it was not surprising in our current findings that smaller ACL volume in both sexes was associated with greater AKL. A previous computational study indicated that smaller ACL graft size lead to greater strain on the ligament during loading (Westermann et al., 2013) with an animal study also reporting that smaller graft ACL volume had lower failure loads.(Fleming et al., 2011) These findings collectively suggest that smaller ACL volume is less capable of resisting external forces, thus likely more lax.

Given ligaments are composed of diverse materials, the various intrinsic properties of these materials could potentially affect function of the ligament.(Culav et al., 1999; Liu et al., 1995; Nakamura et al., 2000; Nordin & Frankel, 1989; Quapp & Weiss, 1997; Raleigh & Collin, 2012) Regardless of ACL volume contribution, lower T2 relaxation times in females and higher T2* relaxation time in males were independently related to greater AKL. Thus indicating that intrinsic ACL properties assessed via T2 and T2* relaxation times are associated with ligamentous function in females and males, respectively. Percentages of water content, collagen density, collagen alignment, and proteoglycan content impacts T2 and T2* relaxation times in articular cartilage.(Li et al., 2011; Mosher et al., 2000; Regatte et al., 2002; Wayne et al., 2003; White et al., 2006) Shorter relaxation times could reflect greater collagen density, a more organized collagen structure, and less water content, while higher relaxation times could reflect lesser

collagen density, a less organized collagen structure, and more free water content.(Matzat et al., 2013) Given that lower ligamentous fibril density was related to weaker cadaver ACLs(Hashemi, Chandrashekar, Mansouri, et al., 2008), these findings indicate that a poorly-structured and disorganized matrix of the ligament is less capable to restrain external loadings, thus resulting in a greater amount of laxity. Hence, it was expected that higher T2* relaxation times would be correlated with a greater AKL in males. Conversely, it was surprising that shorter T2 relaxation time in females was related to greater AKL.

Both T2 and T2* relaxation are sensitive to water content, collagen fiber concentration, collagen orientation, and proteoglycan content.(Li et al., 2011; Mosher et al., 2000; Regatte et al., 2002; Wayne et al., 2003; White et al., 2006) While T2 relaxation looks only at the signal decay resulting from the interaction of protons in water molecules in the tissue, T2* relaxation accounts for both signal decay resulting from protons' interaction within the tissue as well as the interaction with external factors that affect signal decay, such as inconsistencies in the magnetic field or chemical effects on the field.(Pooley, 2005) Realistically, local magnetization is not completely stable which results in rapid signal loss due to diversity in precession angles of protons.(Jung & Weigel, 2013) Therefore, T2* is shorter than T2 relaxation times. Further, reports of T2* relaxation times in ligamentous tissue (Biercevicz, Akelman, et al., 2014; Biercevicz, Akelman, Rubin, et al., 2015) are more common than those of T2. (Fleming et al., 2011)

The current study revealed that T2 and T2* relaxation times could be indicative of intrinsic ACL properties, albeit with opposite directionality that is not well supported by

current literature. Several studies have investigated the correlation between T2 and T2* relaxation times in cartilage measures.(T. Kim et al., 2014; Mamisch et al., 2012; Welsch et al., 2008; Welsch et al., 2010) Multiple in vivo studies report positive associations between T2 and T2*, (Mamisch et al., 2012; Welsch et al., 2008; Welsch et al., 2010) while an in vitro study reported a negative relationship between T2 and T2* relaxation times.(T. Kim et al., 2014) Thus there does not appear to be a consensus of the relationship between T2 and T2* relaxation times and the physiologic environment may alter this relationship. It is critical to note that the relationship between T2 and T2*relaxation times in ligaments is unknown in vitro or in vivo. While greater glycosaminoglycan (Wei et al., 2015), proteoglycan (Wayne et al., 2003), and collagen content (Menezes, Gray, Hartke, & Burstein, 2004) are associated with lower cartilage T2 relaxation times, how these structural components may differentially affect T2* relaxation time of ligaments is poorly understood. It is possible that T2 and T2* relaxation times may be individually more sensitive to differing ligamentous ultrastructure characteristics between males and females. Future sex histologic comparisons in vivo or in vitro in ligaments are needed to clarify these relationships.

When comparing measures of ACL volume, ACL width, and ACL cross-sectional area, they appear to provide different degrees of information with regard to tissue function. Our secondary findings were that smaller ACL volume was the strongest morphometric predictor of greater AKL in both males (r=-.528, P =.017) and females (r=-.683, P =.001). ACL width is a one dimensional measure as calculated by the distance crossing the ACL.(Anderson et al., 2001) This single sagittal plane image may not fully

representative of ligament function. Similar to ACL width, ACL cross-sectional area relies on characterizing ACL morphometry from a single oblique plane.(Whitney et al., 2014) Although both measures are time efficient, they are reliant on the contrast of ligamentous tissue to surrounding tissue in a single plane and do not take into account the full non-uniform, three dimensional nature of the ACL. Thus, the three dimensional measure of ACL volume appears to provide the strongest measure of structure as it related to function.(Chaudhari et al., 2009; Jamison et al., 2010; Whitney et al., 2014)

Given previous studies reported that smaller intercondylar notch was associated with smaller ACL size, (Charlton et al., 2002; Davis et al., 1999; Dienst et al., 2007) our study controlled for femoral notch width when attempting to determine the ACL morphometric measure most predictive of AKL. Our findings indicated that after accounting for femoral notch width, ACL volume was still a predictor of AKL in females ($R^2\Delta$=25%, PΔ=.012), but not in males ($R^2\Delta$=9%, PΔ=.149). After adding ACL volume to the regression model of females, the partial correlation of femoral notch width decreased from -.466 (P = .039) to -.033 (P =.894), indicating that regardless of femoral notch width, ACL volume had the strongest association with AKL in females. Similarly in males, the partial correlation of femoral notch width decreased from -.460 (P = .041) to -.194 (P =.427). This suggests that ACL volume and femoral notch width shared considerable predicted variance in AKL. It also should be noted that the standard deviation of mean in females was 42% higher than in males. The greater variance in females' AKL that was a function of sampling to ensure a wide range of laxity values may have enhanced the predictive ability of ACL volume to AKL in females.

While increased AKL has been prospectively identified as a risk factor of ACL injury,(Branch et al., 2010; Myer et al., 2008; Uhorchak et al., 2003; Woodford-Rogers et al., 1994) this risk factor is traditionally considered non-modifiable. Our current findings enhance the foundational understanding of the clinical AKL measure. Better understanding the contributions of intrinsic ACL properties to AKL could advance the future prevention efforts that focus on increasing ligament strength, and potentially lower AKL. Further, our study also provided sex-specific models of how the ACL morphometry and structural composition combine to predict AKL. These findings could contribute to better identification of the underlying intrinsic ACL injury risk factors and the corresponding clinical measure in order to better address the sex bias on ACL injury.

A limitation of the current study was that all ACL morphometry measures were obtained via manually segmentation. Based on the resolution of the sequence, the chosen image, and the subject variation; there is an expected degree of measurement error between and within participants. However, intra-tester consistency and precision measures has been established. A second limitation was that all our measures were obtained from the left knee. This decision was based on previous reports of high degrees of AKL and ACL volume symmetry between limbs.(Jamison et al., 2010; Shultz & Nguyen, 2007) The symmetry of ligamentous T2 and T2* relaxation times is unknown. Third, while the study was based upon obtaining a wide range of AKL in males and females, the wide included range of physical activity level may confound the current findings and hurt external validity to populations at high risk of non-contact ACL injury. However, the recruited wider range of populations could help us to better drive reliable

estimates of understanding the gross relationship of ACL morphometry and structural composition to AKL.

Conclusion

Primary current main findings were that smaller ACL volume combined with lower T2 relaxation times in females and smaller ACL volume combined with higher T2* relaxation times in males to predict greater AKL. The secondary findings were that ACL volume was the strongest predictor of AKL in both sexes. These findings collectively indicated that ACL morphometric and structural composition factors independently and collectively associated with AKL. Contrary to our hypothesis, the finding that shorter T2 relaxation times in females was predictive of ligament function warrants further study as to the in-vivo relationship of T2 and T2*relaxation times to ligamentous structural properties. Future studies should continue to address factors associated with increased AKL to help focus future intervention efforts to enhance the ligament strength and reduce laxity.

Table 6.1 Participants' Descriptive Statistics (Mean ± Standard Deviation)

	Males (N=20)	Females (N=20)
Age (yrs)	23.3 ± 2.9	21.3 ± 2.3
Height (cm)	180.4 ± 6.7	166.9 ± 7.7
Weight (kg)	84.0 ± 10.9	61.9 ± 7.2
Activity-Rating Score	9.2 ± 4.1	10.7 ± 3.9
AKL (mm)	6.3 ± 1.9	8.1 ± 2.7
ACL Width (mm)	8.5 ± 2.3	7.0 ± 1.2
ACL Volume (mm^3)	1712.2 ± 356.3	1200.1 ± 337.8
ACL CSA (cm^2)	0.9 ± 0.2	0.8 ± 0.2
Femoral Notch Width(mm)	19.1 ± 1.8	16.5 ± 1.1
T2 (ms)	57.5 ± 8.2	55.9 ± 8.1
T2* (ms)	19.1 ± 2.5	18.5 ± 2.2

Table 6.2 Obtained AKL Distributions

	Males	**Females**
Below Average AKL	4.1 ± 0.4 (N=6)	5.0 ± 0.4 (N=6)
Average AKL	6.1 ± 0.5 (N=6)	7.9 ± 1.3 (N=8)
Above Average AKL	8.1 ± 1.4 (N=6)	11.4 ± 0.6 (N=6)

Table 6.3 Bivariate Correlations of All Variables

Males	**ACL_V**	**ACL_W**	**ACL_CSA**	**FNW**	**T2**	**T2***
AKL	-.53*	-.42	-51*	-.46*	-.18	.38
ACL_Volume		.73*	.66*	.63*	.14	.03
ACL_Width			.59*	.40	.20	.12
ACL_Cross-sectional area (CSA)				.59*	-.04	.30
Femoral Notch Width (FNW)					.18	.08
T2 relaxation times						.14
T2* relaxation times						1
Females	**ACL_V**	**ACL_W**	**ACL_CSA**	**FNW**	**T2**	**T2***
AKL	-.68*	-.42	-54*	-.47*	-.52*	-.18
ACL_Volume		.36	.71*	.66*	.07	.35
ACL_Width			.70*	.42	.28	.13
ACL_Cross-sectional area (CSA)				.77*	.16	.25
Femoral Notch Width (FNW)					.21	.25
T2 relaxation times						-.12
T2* relaxation times						1

* Significant correlation between variables (P≤.05)

Table 6.4 Stepwise Regression Model of Femoral Notch Width and ACL Volume Predicting AKL

Model (Female)	R^2	$R^2\Delta$	Sig. FΔ
1	.217	.217	.039
2	.467	.251	.012
Model (Male)			
1	.212	.212	.041
2	.305	.093	.149

Model1 Predictors: (Constant), femoral notch width
Model2 Predictors: (Constant), femoral notch width, ACL volume

Table 6.5 Stepwise Regression Coefficients and Correlations of Femoral Notch Width and ACL Volume Predicting AKL

Females				Correlations		
Coefficients	Beta	t	Sig.	Zero-order	Partial	Part
(Constant)	15.631	2.005	.061			
Femoral notch width	-.074	-.135	.894	-.466	-.033	-.024
ACL volume	-.005	-2.828	.012	-.683	-.566	-.501
Males				Correlations		
Coefficients	Beta	t	Sig.	Zero-order	Partial	Part
(Constant)	14.218	3.397	.003			
Femoral notch width	-.224	-.814	.427	-.460	-.194	-.165
ACL volume	-.002	-1.512	.149	-.528	-.344	-.306

Table 6.6 Stepwise Regression Model of T2 and T2* Relaxation Times Predicting AKL

Model (Female)	R^2	$R^2\Delta$	Sig. FΔ
1	.265	.265	.020
2	.324	.059	.239
Model (Male)			
1	.145	.145	.098
2	.201	.057	.287

Model1 Predictors: (Constant), T2 relaxation times in female, T2* relaxation times in male
Model2 Predictors: (Constant), T2* relaxation times in female, T2 relaxation times in males

Table 6.7 Stepwise Regression Coefficients and Correlations of T2 and T2* Relaxation Times Predicting AKL

Females				Correlations		
Coefficients	Beta	t	Sig.	Zero-order	Partial	Part
(Constant)	23.610	3.828	.001			
T2 relaxation times	-.181	-2.707	.015	-.515	-.549	-.540
T2* relaxation times	-.294	-1.220	.239	-.181	-.284	-.243
Males				Correlations		
Coefficients	Beta	t	Sig.	Zero-order	Partial	Part
(Constant)	3.385	.827	.420			
T2* relaxation times	.323	1.895	.075	.381	.418	.411
T2 relaxation times	-.056	-1.099	.287	-.181	-.257	-.238

Table 6.8 Stepwise Regression Model of ACL Volume and T2 or T2* Relaxation Times Predicting AKL

Model (Female)	R^2	$R^2\Delta$	Sig. FΔ
1	.467	.467	.001
2	.684	.217	.003
Model (Male)			
1	.278	.278	.017
2	.437	.158	.043

Model1 Predictors: (Constant), ACL volume
Model2 Predictors in Females: (Constant), ACL volume, T2 relaxation times
Model2 Predictors in Males: (Constant), ACL volume, and T2* relaxation time

Table 6.9 Stepwise Regression Coefficients and Correlations Model of ACL Volume and T2 or T2* Relaxation Times Predicting AKL

Females				Correlations		
Coefficients	Beta	t	Sig.	Zero-order	Partial	Part
(Constant)	22.963	8.203	.000			
ACL volume	-.005	-4.742	.000	-.683	-.755	-.647
T2 relaxation times	-.155	-3.412	.003	-.515	-.638	-.466
Males				Correlations		
Coefficients	Beta	t	Sig.	Zero-order	Partial	Part
(Constant)	5.381	1.703	.107			
ACL volume	-.003	-2.968	.009	-.528	-.584	-.540
T2* relaxation times	.310	2.186	.043	.381	.468	.398

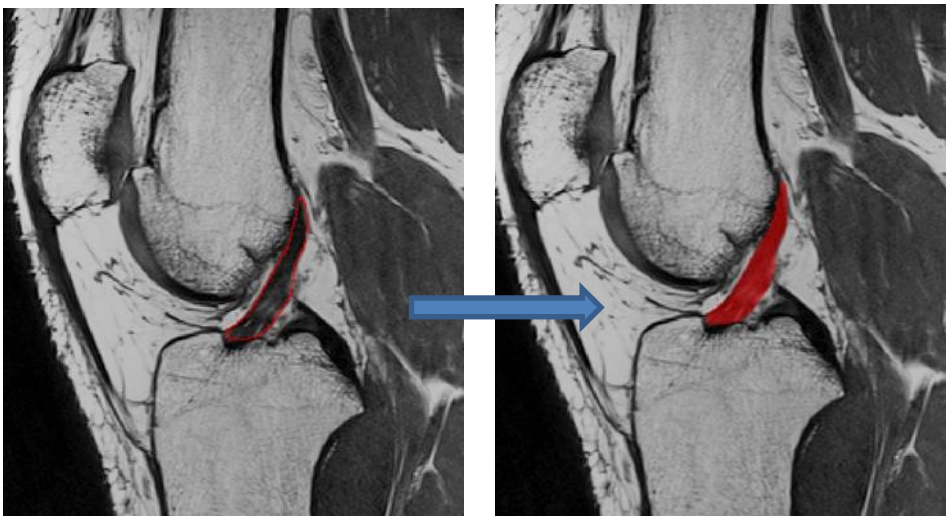

Figure 6.1 ACL Volume Measure. Manual ACL segmentation and resultant area (shaded) on sagittal image.

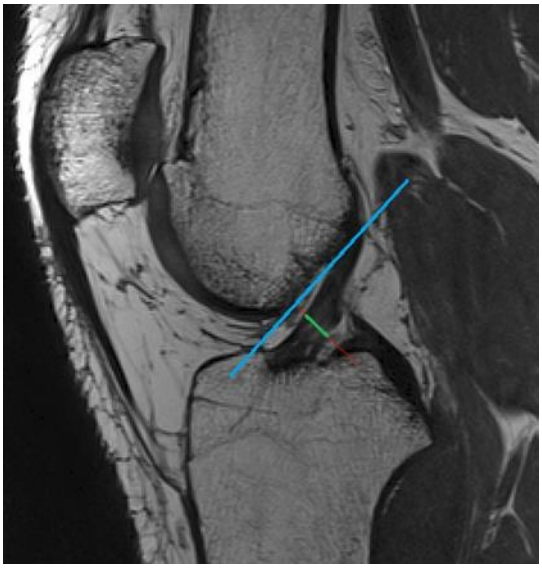

Figure 6.2 ACL Width Measure. The distance across the ACL (green) on a line (red) perpendicular to the Blumensaat's line (blue).

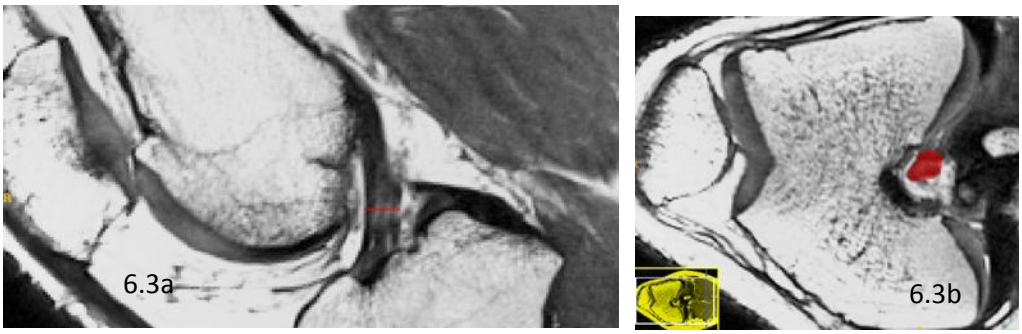

Figure 6.3 CSA Measure. (a) Identified CSA from the oblique sagittal image; (b) The ACL was segmented and CSA calculated from the oblique axial image.

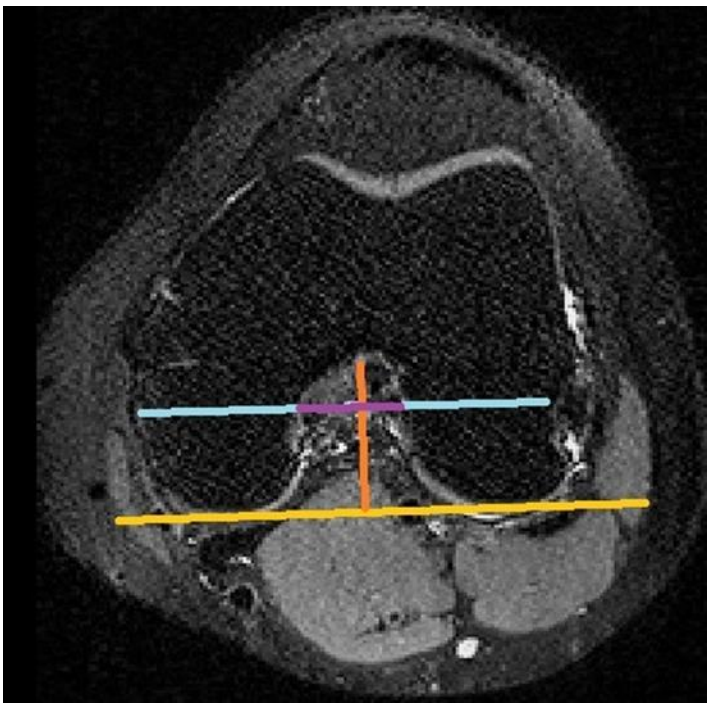

Figure 6.4 Femoral Notch Width Measure. The distance across the notch (purple) taken from a line (blue) parallel to a line tangent to a line located tangent to the posterior femoral condyle located at 2/3 of the notch depth (orange).

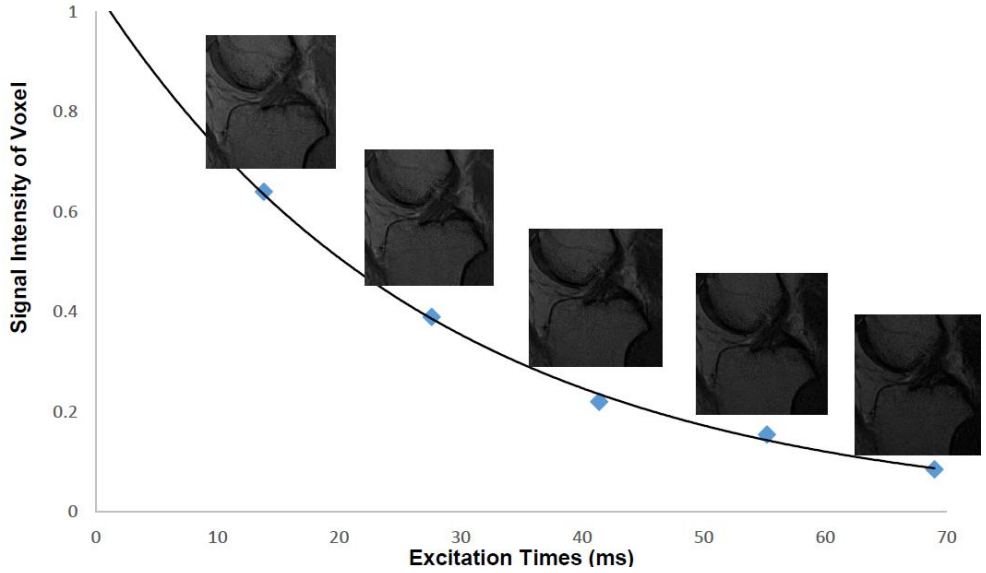

Figure 6.5 Example of T2 Relaxation Time. Example of T2 relaxation time quantification using the voxel-wise data from each of the 5 excitation times. The resultant rate of voxel by voxel signal intensity decay was quantified over the 5 times using the Levenberg-Marquardt monoexpoential equation (Fleming et al., 2011).

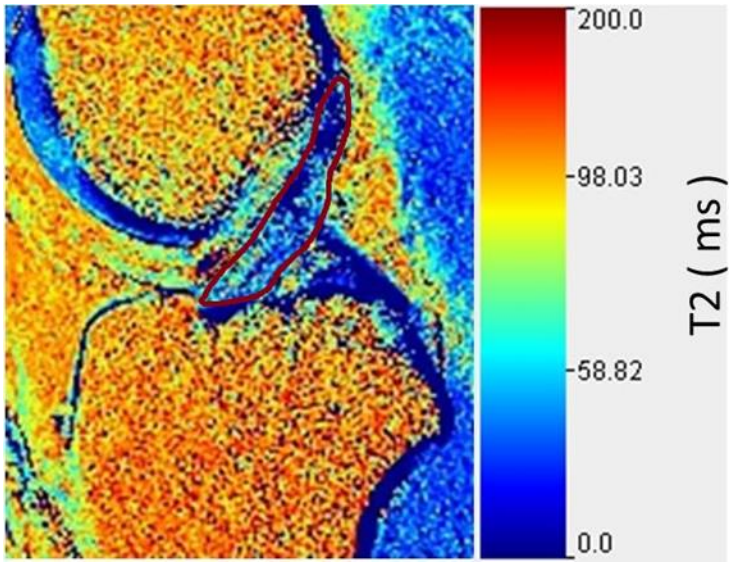

Figure 6.6 Example of T2 Relaxation Map. Example of T2 relaxation map was ACL outlined by brown contour.

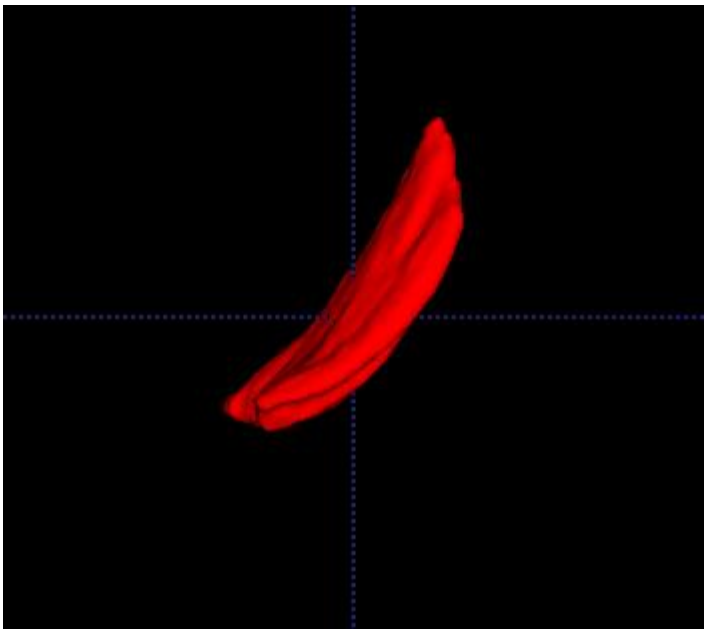

Figure 6.7 3D ACL Model. 3D ACL model ascertained from segmented image.

CHAPTER VII

CONCLUSIONS

A better understanding of how intrinsic ligamentous characteristics associate with ACL function can positively impact our understanding of ACL injury. This study examined the extent to which in vivo measures of ACL morphometry and structural composition collectively predicted AKL in active healthy males and females. A clinical goal was to better understand the factors contributing to greater AKL in order to advance future intervention/prevention efforts focused on enhancing ligament strength. Both ACL morphometry and MRI relaxation times measures associated with structural composition were predictive of AKL. Although previous studies (Grood et al., 1992; H.-M. Wang et al., 2015) reported that smaller ACL morphometry was associated with greater AKL, the current investigation is the first in vivo report of the structural composition of the ligament also being related to AKL. However, it is still unknown as to how such intrinsic ligamentous characteristics are associated with ACL injury risk.

The majority of recent research investigating risk factors of ACL injury has revolved around biomechanical strategies as these studies offer an avenue of high potential for intervention/prevention. However, the literature is somewhat mixed with regard to the effectiveness of movement biomechanics to predict injury risk. (Hewett et al., 2005; Padua et al., 2015; Smith et al., 2012) Inappropriate landing biomechanics could produce a high external loadings on the ACL, (B. Yu & Garrett, 2007) thus

increasing the potential for ACL rupture. However, this biomechanical prospective may not fully explain the likelihood of ACL injury as the magnitude of external loadings needed to rupture the ACL could vary depending on intrinsic ligamentous characteristics indicative of ligamentous strength. (Fleming et al., 2011) Due to the perceived difficulty in intervening upon such factors, anatomic factors are often dismissed. However, recent evidence shows great promise in the usefulness of such factors to predict noncontact ACL injury risk.(Whitney et al., 2014) Investigation of anatomic factors provides insight to a comprehensive prediction model for assessing injury risk. While direct intervention of anatomical factors may not always be feasible, development of interventions to moderate the risk of injury in individuals with high-risk anatomical factors may be of clinical importance.

Although that various ligamentous structural characteristics have a potential to influence ligament function, how these intrinsic factors collectively contributing to increased injury risk is unknown. While invasive in vitro techniques could provide histological evidences of the relationship of ligamentous structural properties to corresponding to ligamentous biomechanics, in-vitro results may not be fully externally valid to the in vivo setting. Further, the findings from animal in vivo studies have some degree of challenges such as biological function, biomechanical responses, and structural composition differences compared to humans. To this point, the in vivo structural composition of human ligaments is relatively unknown.

Recent advances in quantitative MRI have provided plausible measures for assessing in vivo ligamentous structural composition. MRI is a non- invasive method to

detect the signal intensity changes from hydrogen atoms in order to create diagnostic images.(Pooley, 2005) Due to MRI signal intensity sensitivity to free hydrogen distribution, (Fullerton & Rahal, 2007) the rate of signal decay could be indicative of differences in collagen structure of ligaments. (Biercevicz, Proffen, Murray, Walsh, & Fleming, 2015) T2 and T2* relaxation imaging may have the ability to assess structural composition of the ligament. (Biercevicz, Akelman, et al., 2014; Biercevicz, Akelman, Rubin, et al., 2015; Fleming et al., 2011) While this approach has been recently utilized in the vivo animal grafts, (Fleming et al., 2011) as well as cadaver ligaments (Biercevicz, Akelman, Rubin, et al., 2015), the ability of such imaging techniques to assess healthy, in vivo ligament were unknown to this point.

This investigation utilized the novel methods of T2 and T2* to evaluate the relationship of ligamentous structural composition to the ligamentous function measure of AKL. Based on T2 and T2* relaxation times sensitivities to water and collagen in cartilage, (Li et al., 2011; Mosher et al., 2000; Regatte et al., 2002; Wayne et al., 2003; White et al., 2006) T2 and T2* could be applied to indicate ligamentous structural composition. (Biercevicz, Akelman, et al., 2014; Biercevicz, Akelman, Rubin, et al., 2015; Fleming et al., 2011) Our study indicated after accounting for ACL volume, lower T2 in females and higher T2* in males predicted greater AKL. It was expected that higher T2* in males, which suggests less collagen density, disorganized collagen matrix, and more free water distribution, could predict grater AKL. However, the directionality of the females' relationship of relaxation time to AKL conflicted with the limited work that exists in this area. Additionally, our current measures of structural composition, T2 and

T2* relaxation times, did not differ between sexes while other cadaver based histologic studies have demonstrated sex differences. (Hashemi, Chandrashekar, Mansouri, et al., 2008) Hence, further sex comparisons of structural composition as assessed via T2 and T2* in vitro and vivo is warranted.

The sex disparity in ACL injury rates has been repeatedly reported,(Beynnon et al., 2014; Prodromos et al., 2007) but the underlining reasons are still uncertain. The larger ACL morphometry in males may offer some insight as ACL volume has been prospectively associated with ACL injury risk. (Whitney et al., 2014) Due to the better delineation of ACL morphometry and representative of ACL anatomy, ACL volume appears to be the most appropriate morphometric measure to use in structural studies of ACL biomechanics and injury. However, the factors that may directly contribute to ACL morphometry are not well understood.

The investigations of ACL injury risk have been divided in groups such as biomechanical, hormonal, genetic and anatomical injury risk factors. Little is understood how these injury risk factors interact to each other. Ligamentous intrinsic characteristics such as ACL morphology and structural composition could be indicative of weak or strong ligaments and related to ligamentous function. While biomechanical injury factors have focused on reducing external loadings, we do not know if the mechanism of non-contact ACL injury is one-time even or if it is the result of chronic loading patterns over time (Shultz, Schmitz, et al., 2012). Hence, future study should include combinations of biomechanical and intrinsic factors to advance our ability to prediction and intervene upon injury risk factors. Further, investigations of hormonal and genetic factors on

intrinsic characteristics may contribute the underlining mechanism(s) of sex disparity and family traits of ACL injury. This direction of research could provide for comprehensive prediction models, comprehensive intervention design, and optimized rehabilitation, to most positively impact joint health.

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APPENDIX A

PHYSICAL ACTIVITY AND HEALTH HISTORY

Do you have any General Health Problems or Illnesses? (e.g. diabetes, respiratory disease) Yes____ No____

Do you have any vestibular (inner ear) or balance disorders? Yes____ No____

Do you smoke? Yes____ No____

Do you drink alcohol? Yes____ No____ If yes, how often?_____

Do you have any history of connective tissue disease or disorders? (e.g. Ehlers-Danlos, Marfan's Syndrome, Rheumatoid Arthritis) Yes____ No____

Has a family member of yours ever been diagnosed with breast cancer? Yes____ No____ (if no, please skip next question.)

If yes, please put a check next to the types of relatives that have been diagnosed. You may check more than one box:

Mother_____ Sister_____ Grandmother _____ Aunt _____.
Male relative (father, brother, grandfather, or uncle) _____.
Other type of relative (please write in) _____.

Please list any medications you take regularly: _____

_____

Please list any previous injuries to your lower extremities. Please include a description of the injury (e.g. ligament sprain, muscle strain), severity of the injury, date of the injury, and whether it was on the left or right side.

Body Part	Description	Severity	Date of Injury	L or R
Hip				
Thigh				
Knee				

Lower Leg

Ankle

Foot

Please list any previous surgery to your lower extremities (Include a description of the surgery, the date of the surgery, and whether it was on the left or right side)

Body Part	Description	Date of Surgery	L or R

Please list all physical activities that you are currently engaged in. For each activity, please indicate how much time you spend each week in this activity, the intensity of the activity (i.e. competitive or recreational) and for how long you have been regularly participating in the activity.

Activity	#Days/week	#Minutes/Day	Intensity	Activity Began When?

What time of day do you generally engage in the above activities?

Please list other conditions / concerns that you feel we should be aware of:

The Activity Rating Scale

Please indicate how often you performed each activity in your healthiest and most active state, **in the past year.**

	Less than one time in a month	**One time in a month**	**One time in a week**	**2 or 3 times in a week**	**4 or more times in a week**
Running: running while playing a sport or jogging					
Cutting: Changing directions while running					
Decelerating: coming to a quick stop while running					
Pivoting: turning your body with your foot planted while playing a sport; For example: skiing, skating, kicking, throwing, hitting a ball (golf, tennis, squash), etc.					

Investigator Comments:

APPENDIX B

PHYSICAL ACTIVITY QUESTIONNAIRE

In this section, we would like to ask you about your current sport/physical activity/exercise habits that you perform regularly. Please answer the following questions as accurately as possible. When answering, consider the definitions of strenuous, moderate, and mild exercise (listed below).

STRENUOUS EXERCISE (HEART BEATS RAPIDLY): e.g.- running/jogging/elliptical at vigorous pace, vigorous swimming, vigorous long distance bicycling, heavy lifting etc.
MODERATE EXERCISE (NOT EXHAUSTING): e.g.- fast walking/jogging at moderate pace, easy bicycling, easy swimming, weight training, etc.
MILD EXERCISE (MINIMAL EFFORT): e.g.- casual walking, stretching, light resistance exercises, etc.

1.) **During a typical 7-Day period (a week), how many times on the average do you participate in <u>strenuous</u> exercise for more than 20 minutes**?

Average # of times/week

Please list <u>strenuous</u> physical activities that your participate in regularly.

2.) **During a typical 7-Day period (a week), how many times on the average do you participate in <u>moderate</u> exercise for more than 20 minutes?**

Average # of times/week

Please list <u>moderate</u> physical activities that your participate in regularly.

3.) **During a typical 7-Day period (a week), how many times on the average do you participate in <u>mild</u> exercise for more than 20 minutes?**

Average # of times/week

Please list the <u>mild</u> physical activities that your participate in regularly.

APPENDIX C

MRI FULL SCAN SEQUENCE

SIEMENS MAGNETOM TrioTim syngo MR B17

\\USER\knee\Schmitz\Spring 2015 structural mapping finalL\Loc left 300mm (3-plane, sag, cor,axial)
TA: 7.5 s PAT: 2 Voxel size: 2.3×2.3×3.0 mm Rel. SNR: 1.00 SIEMENS: tse

Properties	
Prio Recon	Off
Before measurement	
After measurement	
Load to viewer	On
Inline movie	Off
Auto store images	On
Load to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Start measurement without further preparation	Off
Wait for user to start	Off
Start measurements	single

Routine	
Slice group 1	
Slices	1
Dist. factor	10 %
Position	L200.0 P0.0 H0.0
Orientation	Sagittal
Phase enc. dir.	A >> P
Rotation	0.00 deg
Slice group 2	
Slices	1
Dist. factor	10 %
Position	Isocenter
Orientation	Coronal
Phase enc. dir.	R >> L
Rotation	0.00 deg
Slice group 3	
Slices	1
Dist. factor	10 %
Position	Isocenter
Orientation	Transversal
Phase enc. dir.	A >> P
Rotation	0.00 deg
Phase oversampling	50 %
FoV read	300 mm
FoV phase	100.0 %
Slice thickness	3.0 mm
TR	500 ms
TE	7.5 ms
Averages	1
Concatenations	1
Filter	Distortion Corr.(2D), Elliptical filter
Coil elements	K15

Contrast	
MTC	Off
Magn. preparation	None
Flip angle	150 deg
Fat suppr.	None
Water suppr.	None
Restore magn.	Off
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	1
Multiple series	Each measurement

Resolution	
Base resolution	128
Phase resolution	100 %
Phase partial Fourier	Off
Trajectory	Cartesian
Interpolation	Off
PAT mode	GRAPPA
Accel. factor PE	2
Ref. lines PE	27
Matrix Coil Mode	Auto (Triple)
Reference scan mode	Integrated
Image Filter	Off
Distortion Corr.	On
Mode	2D
Unfiltered images	Off
Prescan Normalize	Off
Normalize	Off
B1 filter	Off
Raw filter	Off
Elliptical filter	On
Mode	Inplane

Geometry	
Multi-slice mode	Interleaved
Series	Interleaved
Special sat.	None
Tim CT mode	Off

System	
Body	Off
K15	On
Positioning mode	ISO
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	H >> F
Save uncombined	Off
Coil Combine Mode	Adaptive Combine
Auto Coil Select	Default
Shim mode	Tune up
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Silicone	Off
? Ref. amplitude 1H	0.000 V
Adjustment Tolerance	Auto
Adjust volume	
Position	Isocenter
Orientation	Transversal
Rotation	0.00 deg
R >> L	350 mm
A >> P	263 mm
F >> H	350 mm

Physio	
1st Signal/Mode	None
Dark blood	Off
Resp. control	Off

Inline	
Subtract	Off
Std-Dev-Sag	Off

Std-Dev-Cor	Off
Std-Dev-Tra	Off
Std-Dev-Time	Off
MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Sequence

Introduction	On
Dimension	2D
Compensate T2 decay	Off
Reduce Motion Sens.	Off
Contrasts	1
Bandwidth	250 Hz/Px
Flow comp.	No
Allowed delay	60 s
Echo spacing	7.49 ms
Define	Turbo factor
Turbo factor	10
Echo trains per slice	11
RF pulse type	Fast
Gradient mode	Fast

SIEMENS MAGNETOM TrioTim syngo MR B17

\\USER\knee\Schmitz\Spring 2015 structural mapping finalL\Left_Anatomical Localizer 160mm (3-plane, sag,

TA: 0:24 PAT: 2 Voxel size: 1.2×1.2×3.0 mm Rel. SNR: 1.00 SIEMENS: tse

Properties

Prio Recon	Off
Before measurement	
After measurement	
Load to viewer	On
Inline movie	Off
Auto store images	On
Load to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Start measurement without further preparation	On
Wait for user to start	On
Start measurements	single

Routine

Slice group 1	
Slices	1
Dist. factor	10 %
Position	L100.0 P0.0 H0.0
Orientation	Sagittal
Phase enc. dir.	A >> P
Rotation	0.00 deg
Slice group 2	
Slices	1
Dist. factor	10 %
Position	Isocenter
Orientation	Coronal
Phase enc. dir.	R >> L
Rotation	0.00 deg
Slice group 3	
Slices	1
Dist. factor	10 %
Position	Isocenter
Orientation	Transversal
Phase enc. dir.	A >> P
Rotation	0.00 deg
Phase oversampling	50 %
FoV read	300 mm
FoV phase	100.0 %
Slice thickness	3.0 mm
TR	1000 ms
TE	30.0 ms
Averages	1
Concatenations	1
Filter	Distortion Corr.(2D), Elliptical filter
Coil elements	K15

Contrast

MTC	Off
Magn. preparation	Slice-sel. IR
TI	220 ms
Freeze suppressed tissue	Off
Flip angle	150 deg
Fat suppr.	None
Water suppr.	None
Restore magn.	Off
Averaging mode	Long term
Reconstruction	Magnitude
Measurements	1
Multiple series	Each measurement

Resolution

Base resolution	256
Phase resolution	100 %
Phase partial Fourier	Off
Trajectory	Cartesian
Interpolation	Off
PAT mode	GRAPPA
Accel. factor PE	2
Ref. lines PE	35
Matrix Coil Mode	Auto (Triple)
Reference scan mode	Integrated
Image Filter	Off
Distortion Corr.	On
Mode	2D
Unfiltered images	Off
Prescan Normalize	Off
Normalize	Off
B1 filter	Off
Raw filter	Off
Elliptical filter	On
Mode	Inplane

Geometry

Multi-slice mode	Interleaved
Series	Interleaved
Special sat.	None
Tim CT mode	Off

System

Body	Off
K15	On
Positioning mode	ISO
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	H >> F
Save uncombined	Off
Coil Combine Mode	Adaptive Combine
Auto Coil Select	Default
Shim mode	Tune up
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Silicone	Off
? Ref. amplitude 1H	0.000 V
Adjustment Tolerance	Auto
Adjust volume	
Position	Isocenter
Orientation	Transversal
Rotation	0.00 deg
R >> L	350 mm
A >> P	263 mm
F >> H	350 mm

Physio

1st Signal/Mode	None
Dark blood	Off
Resp. control	Off

SIEMENS MAGNETOM TrioTim syngo MR B17

Inline

Subtract	Off
Std-Dev-Sag	Off
Std-Dev-Cor	Off
Std-Dev-Tra	Off
Std-Dev-Time	Off
MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Sequence

Introduction	On
Dimension	2D
Compensate T2 decay	Off
Reduce Motion Sens.	Off
Contrasts	1
Bandwidth	250 Hz/Px
Flow comp.	No
Allowed delay	60 s
Echo spacing	7.49 ms
Define	Turbo factor
Turbo factor	10
Echo trains per slice	21
RF pulse type	Fast
Gradient mode	Fast

SIEMENS MAGNETOM TrioTim syngo MR B17

\\USER\knee\Schmitz\Spring 2015 structural mapping finalL\pd_spc_rst_fs_sag_p2_iso_320

TA: 8:29 PAT: 2 Voxel size: 0.5×0.5×0.6 mm Rel. SNR: 1.00 SIEMENS: tse_vfl

Properties

Prio Recon	Off
Before measurement	
After measurement	
Load to viewer	On
Inline movie	Off
Auto store images	On
Load to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Start measurement without further preparation	On
Wait for user to start	On
Start measurements	single

Routine

Slab group 1	
Slabs	1
Position	Isocenter
Orientation	Sagittal
Phase enc. dir.	A >> P
Rotation	0.00 deg
Phase oversampling	0 %
Slice oversampling	0.0 %
Slices per slab	176
FoV read	160 mm
FoV phase	100.0 %
Slice thickness	0.60 mm
TR	1200 ms
TE	33 ms
Averages	1.4
Concatenations	1
Filter	Raw filter, Prescan Normalize
Coil elements	K15

Contrast

MTC	Off
Magn. preparation	None
Fat suppr.	SPAIR
Fat sat. mode	Strong
Water suppr.	None
Restore magn.	On
Reconstruction	Magnitude
Measurements	1
Multiple series	Off

Resolution

Base resolution	320
Phase resolution	96 %
Slice resolution	92 %
Phase partial Fourier	Off
Slice partial Fourier	5/8
Interpolation	Off
PAT mode	GRAPPA
Accel. factor PE	2
Ref. lines PE	24
Accel. factor 3D	1
Matrix Coil Mode	Auto (Triple)
Reference scan mode	Integrated
Image Filter	Off
Distortion Corr.	Off
Unfiltered images	Off
Prescan Normalize	On
Normalize	Off
B1 filter	Off
Raw filter	On
Intensity	Weak
Slope	25
Elliptical filter	Off

Geometry

Sat. region 1	
Thickness	80 mm
Position	Isocenter
Orientation	C > T-30.0
Special sat.	None

System

Body	Off
K15	On
Positioning mode	REF
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	H >> F
Save uncombined	Off
Coil Combine Mode	Adaptive Combine
Auto Coil Select	Default
Shim mode	Standard
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Silicone	Off
? Ref. amplitude 1H	0.000 V
Adjustment Tolerance	Auto
Adjust volume	
Position	Isocenter
Orientation	Sagittal
Rotation	0.00 deg
F >> H	160 mm
A >> P	160 mm
R >> L	106 mm

Physio

1st Signal/Mode	None
Dark blood	Off
Resp. control	Off

Inline

Subtract	Off
Std-Dev-Sag	Off
Std-Dev-Cor	Off
Std-Dev-Tra	Off
Std-Dev-Time	Off
MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Sequence

Introduction	On
Dimension	3D
Bandwidth	539 Hz/Px

Flow comp.	No
Allowed delay	30 s
Echo spacing	4.6 ms
Adiabatic-mode	Off
Define	Echo trains
Turbo factor	55
Slice turbo factor	1
Echo trains per slice	3
Echo train duration	267
RF pulse type	Normal
Gradient mode	Fast
Excitation	Slab-sel.
Flip angle mode	PD var

SIEMENS MAGNETOM TrioTim syngo MR B17

\\USER\knee\Schmitz\Spring 2015 structural mapping finalL\T2-star Map

TA: 6:58 PAT: Off Voxel size: 0.5×0.5×3.0 mm Rel. SNR: 1.00 SIEMENS: gre

Properties	
Prio Recon	Off
Before measurement	
After measurement	
Load to viewer	On
Inline movie	Off
Auto store images	On
Load to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	On
Start measurement without further preparation	On
Wait for user to start	On
Start measurements	single

Routine	
Slice group 1	
Slices	20
Dist. factor	20 %
Position	Isocenter
Orientation	Sagittal
Phase enc. dir.	A >> P
Rotation	0.00 deg
Phase oversampling	0 %
FoV read	280 mm
FoV phase	81.3 %
Slice thickness	3.0 mm
TR	1000 ms
TE 1	8.26 ms
TE 2	10.28 ms
TE 3	12.30 ms
TE 4	14.32 ms
TE 5	16.34 ms
TE 6	18.36 ms
TE 7	20.38 ms
TE 8	22.40 ms
TE 9	24.42 ms
TE 10	26.44 ms
TE 11	28.46 ms
TE 12	30.48 ms
Averages	1
Concatenations	1
Filter	Distortion Corr.(2D), Prescan Normalize
Coil elements	K15

Contrast	
MTC	Off
Magn. preparation	None
Flip angle	90 deg
Fat suppr.	Fat sat.
Water suppr.	None
SWI	Off
Averaging mode	Short term
Reconstruction	Magnitude
Measurements	1
Multiple series	Off

Resolution	
Base resolution	512
Phase resolution	100 %
Phase partial Fourier	Off
Interpolation	Off
PAT mode	None
Matrix Coil Mode	Auto (CP)
Image Filter	Off
Distortion Corr.	On
Mode	2D
Unfiltered images	Off
Unfiltered images	Off
Prescan Normalize	On
Normalize	Off
B1 filter	Off
Raw filter	Off
Elliptical filter	Off

Geometry	
Multi-slice mode	Interleaved
Series	Interleaved
Saturation mode	Standard
Special sat.	None
Tim CT mode	Off

System	
Body	Off
K15	On
Positioning mode	FIX
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	H >> F
Save uncombined	Off
Coil Combine Mode	Adaptive Combine
Auto Coil Select	Default
Shim mode	Standard
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Silicone	Off
? Ref. amplitude 1H	0.000 V
Adjustment Tolerance	Auto
Adjust volume	
Position	Isocenter
Orientation	Sagittal
Rotation	0.00 deg
F >> H	280 mm
A >> P	228 mm
R >> L	72 mm

Physio	
1st Signal/Mode	None
Segments	1
Dark blood	Off
Resp. control	Off

Inline	
Subtract	Off
Liver registration	Off
Std-Dev-Sag	Off
Std-Dev-Cor	Off
Std-Dev-Tra	Off
Std-Dev-Time	Off
MIP-Sag	Off

MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On
Wash - In	Off
Wash - Out	Off
TTP	Off
PEI	Off
MIP - time	Off

Sequence

Introduction	On
Dimension	2D
Phase stabilisation	Off
Asymmetric echo	Off
Contrasts	12
Bandwidth 1	650 Hz/Px
Bandwidth 2	650 Hz/Px
Bandwidth 3	650 Hz/Px
Bandwidth 4	650 Hz/Px
Bandwidth 5	650 Hz/Px
Bandwidth 6	650 Hz/Px
Bandwidth 7	650 Hz/Px
Bandwidth 8	650 Hz/Px
Bandwidth 9	650 Hz/Px
Bandwidth 10	650 Hz/Px
Bandwidth 11	650 Hz/Px
Bandwidth 12	650 Hz/Px
Flow comp. 1	No
Flow comp. 2	No
Flow comp. 3	No
Flow comp. 4	No
Flow comp. 5	No
Flow comp. 6	No
Flow comp. 7	No
Flow comp. 8	No
Flow comp. 9	No
Flow comp. 10	No
Flow comp. 11	No
Flow comp. 12	No
Readout mode	Bipolar
Allowed delay	0 s
RF pulse type	Normal
Gradient mode	Fast
Excitation	Slice-sel.
RF spoiling	On

SIEMENS MAGNETOM TrioTim syngo MR B17

\\USER\knee\Schmitz\Spring 2015 structural mapping finalL\T2_map

TA: 5:57 PAT: 2 Voxel size: 0.4×0.4×3.0 mm Rel. SNR: 1.00 SIEMENS: se_mc

Properties

Prio Recon	Off
Before measurement	
After measurement	
Load to viewer	On
Inline movie	Off
Auto store images	On
Load to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Start measurement without further preparation	On
Wait for user to start	On
Start measurements	single

Routine

Slice group 1	
Slices	40
Dist. factor	0 %
Position	Isocenter
Orientation	Sagittal
Phase enc. dir.	A >> P
Rotation	0.00 deg
Phase oversampling	0 %
FoV read	160 mm
FoV phase	100.0 %
Slice thickness	3.0 mm
TR	3040 ms
TE 1	13.8 ms
TE 2	27.6 ms
TE 3	41.4 ms
TE 4	55.2 ms
TE 5	69.0 ms
Averages	1
Concatenations	1
Filter	Prescan Normalize
Coil elements	K15

Contrast

MTC	Off
Magn. preparation	None
Flip angle	180 deg
Fat suppr.	None
Water suppr.	None
Averaging mode	Short term
Reconstruction	Magnitude
Measurements	1
Multiple series	Each measurement

Resolution

Base resolution	384
Phase resolution	100 %
Phase partial Fourier	4/8
Interpolation	Off
PAT mode	GRAPPA
Accel. factor PE	2
Ref. lines PE	26
Matrix Coil Mode	Auto (Triple)
Reference scan mode	Integrated
Image Filter	Off
Distortion Corr.	Off
Unfiltered images	Off
Prescan Normalize	On
Normalize	Off
B1 filter	Off
Raw filter	Off
Elliptical filter	Off

Geometry

Multi-slice mode	Interleaved
Series	Interleaved
Special sat.	None

System

Body	Off
K15	On
Positioning mode	FIX
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	H >> F
Save uncombined	Off
Coil Combine Mode	Adaptive Combine
Auto Coil Select	Default
Shim mode	Tune up
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Silicone	Off
? Ref. amplitude 1H	0.000 V
Adjustment Tolerance	Auto
Adjust volume	
Position	Isocenter
Orientation	Transversal
Rotation	0.00 deg
R >> L	350 mm
A >> P	263 mm
F >> H	350 mm

Physio

1st Signal/Mode	None
Dark blood	Off

Inline

Subtract	Off
Liver registration	Off
Std-Dev-Sag	Off
Std-Dev-Cor	Off
Std-Dev-Tra	Off
Std-Dev-Time	Off
MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Sequence

Introduction	On
Contrasts	5
Bandwidth	228 Hz/Px
Allowed delay	0 s
RF pulse type	Low SAR
Gradient mode	Fast

SIEMENS MAGNETOM TrioTim syngo MR B17

\\USER\knee\Schmitz\Spring 2015 structural mapping finalL\T2ACLvol_left_spc_rst_sag_p2_iso_320

TA: 7:31 PAT: 2 Voxel size: 0.5×0.5×0.5 mm Rel. SNR: 1.00 SIEMENS: tse_vfl

Properties

Prio Recon	Off
Before measurement	
After measurement	
Load to viewer	On
Inline movie	Off
Auto store images	On
Load to stamp segments	Off
Load images to graphic segments	Off
Auto open inline display	Off
Start measurement without further preparation	On
Wait for user to start	Off
Start measurements	single

Routine

Slab group 1	
Slabs	1
Position	Isocenter
Orientation	Sagittal
Phase enc. dir.	A >> P
Rotation	0.00 deg
Phase oversampling	0 %
Slice oversampling	0.0 %
Slices per slab	240
FoV read	150 mm
FoV phase	100.0 %
Slice thickness	0.50 mm
TR	1300 ms
TE	39 ms
Averages	1.0
Concatenations	1
Filter	Raw filter
Coil elements	K15

Contrast

MTC	Off
Magn. preparation	None
Flip angle	160 deg
Fat suppr.	None
Water suppr.	None
Restore magn.	On
Reconstruction	Magnitude
Measurements	1
Multiple series	Each measurement

Resolution

Base resolution	320
Phase resolution	86 %
Slice resolution	83 %
Phase partial Fourier	Allowed
Slice partial Fourier	7/8
Interpolation	Off
PAT mode	GRAPPA
Accel. factor PE	2
Ref. lines PE	24
Accel. factor 3D	1
Matrix Coil Mode	Auto (Triple)
Reference scan mode	Integrated
Image Filter	Off
Distortion Corr.	Off
Prescan Normalize	Off
Normalize	Off
B1 filter	Off
Raw filter	On
Intensity	Weak
Slope	25
Elliptical filter	Off

Geometry

Sat. region 1	
Thickness	80 mm
Position	Isocenter
Orientation	C > T-30.0
Special sat.	None

System

Body	Off
K15	On
Positioning mode	FIX
Table position	H
Table position	0 mm
MSMA	S - C - T
Sagittal	R >> L
Coronal	A >> P
Transversal	H >> F
Save uncombined	Off
Coil Combine Mode	Adaptive Combine
Auto Coil Select	Default
Shim mode	Standard
Adjust with body coil	Off
Confirm freq. adjustment	Off
Assume Silicone	Off
? Ref. amplitude 1H	0.000 V
Adjustment Tolerance	Auto
Adjust volume	
Position	Isocenter
Orientation	Sagittal
Rotation	0.00 deg
F >> H	150 mm
A >> P	150 mm
R >> L	120 mm

Physio

1st Signal/Mode	None
Dark blood	Off
Resp. control	Off

Inline

Subtract	Off
Std-Dev-Sag	Off
Std-Dev-Cor	Off
Std-Dev-Tra	Off
Std-Dev-Time	Off
MIP-Sag	Off
MIP-Cor	Off
MIP-Tra	Off
MIP-Time	Off
Save original images	On

Sequence

Introduction	On
Dimension	3D
Bandwidth	504 Hz/Px
Flow comp.	No

Allowed delay	30 s
Echo spacing	4.9 ms
Adiabatic-mode	Off
Define	Echo trains
Turbo factor	75
Slice turbo factor	1
Echo trains per slice	2
Echo train duration	225
RF pulse type	Normal
Gradient mode	Fast
Excitation	Non-sel.
Flip angle mode	Constant