Impact of Sex and Stretch-Shortening Cycle Induced-Fatigue on Limb Stiffness and Limb Stiffness Asymmetry During the Stop-Jump

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Thesis submitted to the faculty of the Virginia Polytechnic Institute and State University
in partial fulfillment of the requirements for the degree of

Master of Science
In
Biomedical Engineering

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May 8th, 2025
Blacksburg, Virginia

Keywords: Limb stiffness, Asymmetry, Landing Mechanics, Fatigue

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Abstract

Sports-related injuries have a 67% incidence of occurring during competitive play, particularly in sports where fatigue is thought to negatively affect the way that the lower extremities attenuate forces being placed on the body.[72] With the overall incidence of anterior cruciate ligament (ACL) injuries increasing, there is a growing incidence of these injuries occurring in the female population.[117,12] Landing mechanics that are more erect, and those that are more asymmetrical, are known to increase the risk of injury, and may be heightened by the onset of fatigue.[32] This study aimed to assess the effect of sex and fatigue induced by the stretch-shortening cycle on lower limb mechanics and asymmetry values in the stop-jump task. The first purpose of the study was to assess the effect of sex and stretch-shortening cycle fatigue on limb stiffness and limb stiffness asymmetry. The second purpose was to assess landing mechanics that are known to be indicators of how forces are being attenuated by the limbs. The components of limb stiffness were also assessed, including the resultant ground reaction force (rGRF) and change in limb length. A significant interaction was found for nondominant limb change in limb length ($p=0.005$), where males showed an increase in the change in limb length, while females showed a decrease following the fatigue protocol. The rGRF of both limbs was also different between pre- and post-fatigue conditions, decreasing in both sexes with the onset of fatigue. Asymmetry values for peak knee flexion angle, absolute value of knee flexion angle at initial contact (IC), and loading rate were also assessed before and after fatigue. Significant interactions for asymmetry values of peak knee flexion angle and absolute value of knee flexion angle at IC indicated that only female participants had an increase in asymmetry of knee flexion at IC and peak knee flexion values after the fatigue protocol. These results suggest that females adopt a more asymmetrical landing strategy than males after fatigue. A significant increase in peak knee flexion was also found for both sexes after fatigue. Thus, the decrease in rGRF may be due to the increase in peak knee flexion, which aids in the attenuation of the forces placed on the body. The results of this study indicate that, with fatigue, female participants may adopt landing strategies that put them at greater risk of sustaining lower extremity knee injuries during sport.

General Audience Abstract

This study looked at how fatigue potentially affects the way males and females move differently when landing. Most sports related injuries happen during competitive matches when the athlete is more tired, which changes how the body is able to withstand impacts when landing.[72] Athletes are experiencing a growing number of Anterior Cruciate Ligament (ACL) injuries, with females experiencing more injuries than male athletes.[117,12] When athletes are more fatigued, the lower body is unable to absorb impact as well, leading to more upright posture and favoring one side of the body over the other, both of which increase injury risk.[32] This study assessed the impact of fatigue on limb stiffness and limb stiffness asymmetry and the measures that are used to calculate stiffness (for example, resultant ground reaction force (rGRF) and change in limb length) to determine related changes due to fatigue as well as differences between males and females. In addition, this study assessed side-to-side differences (asymmetry) in landing mechanics. Study results indicated that males and females responded differently when tested after the fatigue protocol. Males showed an increase in the change in limb length, while females showed a decrease following fatigue. The rGRF of both limbs was different between pre- and post-fatigue conditions, decreasing in both sexes with the onset of fatigue. Asymmetry values for peak knee flexion angle, absolute value of knee flexion angle at initial contact (IC), and loading rate were also assessed before and after fatigue. Peak knee flexion angle increased in both sexes with the onset of fatigue. The decrease in rGRF may be due to the increase in peak knee flexion angle, which aided in the attenuation of the forces placed on the body. Asymmetry values for peak knee flexion angle, absolute value of knee flexion angle at initial contact (IC), and loading rate were assessed before and after fatigue. Significant interactions for asymmetry values of peak knee flexion angle and absolute value of knee flexion angle at IC were found for females between fatigue conditions. In general, these results indicate that females adopt a more asymmetrical landing strategy than males after fatigue. Therefore, following fatigue, female landings put them more at risk of sustaining lower extremity knee injuries during sport.

Acknowledgments

I would first like to express my deepest gratitude to my parents for the unwavering encouragement and support throughout my educational journey. From a young age, you have believed in me and made countless sacrifices to give me the opportunity to succeed. Your love and guidance have laid the foundation of my achievements. I want to thank my siblings, Mitchell and Elisabeth, for being role models to me. You have led by example through your achievements academically, professionally, and personally and your success has been a constant motivation for me to strive to be my best.

I would like to thank Dr. Robin Queen for her guidance on this project and for giving me a chance to learn hands-on in the lab, it is an opportunity that I am greatly thankful for. I would also like to thank my lab-mates for making my last year at Virginia Tech memorable, creating an environment both within the office and at intramural sports that made every day eventful.

Table of Contents

List of Figures

List of Tables

List of Abbreviations

ACL	Anterior Cruciate Ligament
ACLR	Anterior Cruciate Ligament Reconstruction
D	Dominant
FAST	Functional Agility Short-Term
GRF	Ground Reaction Force
IC	Initial Contact
ND	Nondominant
SJ	Stop Jump
SSC	Stretch Shortening Cycle
SJ	Stop Jump

Chapter 1: Introduction

Motivation

Each year, 2.5 million sports-related injuries are presented to an emergency department, with an average of 740,000 of those injuries occurring at the knee. [21] Knee injuries account for 60% of high-school sports-related surgeries and 41% of total sports-related injuries. [22] Knee injuries often require extensive rehabilitation and can be costly to the patient, having an overall mean cost of $1,131 in females and $1,097 in males per injury in 2000. [24] Females, in particular, are more susceptible to knee injuries, being reported to have 4 to 6 times higher risk of major knee injuries over their male counterparts performing the same sports. [21] This susceptibility can be due to multiple factors including anatomic, neuromuscular, and biomechanical differences. [21]

Poor use of mechanics during sports-related movements such as landings can increase the risk of a non-contact injury to the lower extremity. During landings, there is an increased amount of force exerted on the athlete, requiring both coordination and flexibility to attenuate the forces on the lower limbs. [1] Most of the attenuation from ground reaction forces (GRFs) is absorbed by the lower limbs during the eccentric muscle contraction phase and knee kinematics during landings. [2] When an abnormal landing strategy occurs, the attenuated forces are placed on other structures that cannot withstand the amount of biomechanical load being placed on them, resulting in injury to those structures. [25] Additionally, asymmetries within landings can exert more force on one limb, overloading that limb and decreasing the joint stability. Factors that can affect the stability of the knee joint are GRF, joint angles, joint position, and loading rate.

Furthermore, in the context of landing mechanics, fatigue is an important factor to consider during non-contact sports-related injuries, as neurological and musculoskeletal function can be altered when fatigue is present, affecting energy absorption, knee proprioception, and joint laxity. [27] There is a certain amount of strength needed from the hip and knee extensors to create enough power in the eccentric loading phase of the stretch shortening cycle, where fatigue of these extensors may decrease joint stability and lead to injury. [26] As approximately 67% of all sports-related injuries occur during competition where maximal load is exerted on the athlete, it is important to note that this could be related to the presence of lower extremity fatigue. [23] There have also been sex-specific differences within landing mechanics found after fatigue onset, with female athletes landing with a larger vertical GRF, [8,9] larger anterior shear force, [20] increased vertical GRF asymmetry, [9] lesser knee flexion excursion, [9] and decreased peak knee flexion angles than males. [20]

The evaluation of the leg's resistance to deformation that occurs in response to an externally applied GRF is defined as limb stiffness. [1] This metric provides information on the effect of kinetic and kinematic factors, specifically the GRF and the change in limb length during the landing. While research has been done on the effect of limb stiffness on athletic performance, there is still a lack of knowledge about the effect of limb stiffness on lower extremity injury risk. While an increase in stiffness can enhance athletic performance, the amount that can cause injury is unknown. In contrast to athletic performance, it is understood that landings that are considered too "stiff" or have large between limb asymmetry have an increased risk of developing a lower extremity injury, [3] with females having a higher risk, as they are known to have more asymmetry and stiffer landings in athletic tasks such as the drop landing and stop jump. [4,5,6] In addition, the range of limb stiffness that is considered "optimal" for performance has not been sufficiently addressed, although it is known that too little limb stiffness can cause injury to soft tissue while too much limb stiffness can cause injury to bony structures. [26] This study will use an overhead target during the SJ before and after fatigue to create a more game-like task, similar to that in sports. A horizontal component and an external focus will be included to improve the efficacy of the proposed study aims to determine if a sex-fatigue interaction exists for specific landing mechanics measures. The work of this study specifically aims to:

Specific Aim 1: Determine the sex-specific differences in dominant limb stiffness and stiffness symmetry during a stop-jump before and after induced neuromuscular fatigue during the first landing of a SJ with a suspended target.

Hypothesis 1a: There will be a significant interaction between sex and fatigue with female athletes experiencing greater dominant limb stiffness and greater limb stiffness asymmetry during the post-fatigue SJ.

Hypothesis 1b: There will be a significant interaction between sex and fatigue with female athletes experiencing greater GRF and GRF asymmetry.

Specific Aim 2: Determine sex-specific alterations to landing mechanics during a stop-jump before and after induced neuromuscular fatigue during the first landing of a SJ with a suspended target.

Hypothesis 2: There will be a significant interaction between sex and fatigue with female athletes experiencing larger asymmetry values for loading rate, peak knee flexion, and knee flexion at initial contact during the post-fatigue SJ.

Background

Injuries occurring at the knee have become a very prevalent issue in sports, accounting for approximately 40% of all sports-related injuries.[72] It has been reported that internal knee lesions correspond to almost half of these sport-related knee injuries, with Anterior Cruciate Ligament (ACL) injuries having an incidence of 45% of these recorded lesions.[72] Non-contact injuries specifically, occur when there is no physical contact between players at the time of injury and can occur when improper mechanics are used during athletic movements such as jumping or running tasks. As many as 70% of ACL tears are related to sport[60] and occur from non-contact mechanisms[73]. In the United States alone, 127,000 ACL reconstructions are performed every year, being more common in active adolescents and young adults.[31,73] The average incidence of ACL reconstruction (ACLR) in the general population and in the female population has been steadily increasing in recent years, with a 22% increase in total surgeries performed and a 21% increase in ACLR in the female population between 2002 and 2014.[75] The cost of ACLR, as well as the time commitment of rehabilitation, place large burdens on the patient, with the average immediate cost of the surgery being approximately $9,400, and a total healthcare cost of $13,300, including the 6 months of rehabilitation post-operation.[76] Not only do ACL injuries have major cost drawbacks, but the quality of life (QoL) of athletes decreases, regardless of the decision to receive reconstruction surgery.[31] Half of patients who sustain ACL injuries will develop osteoarthritis (OA) within 12-14 years after surgery, playing a role in the decrease in QoL. [31,80]

While increases in ACL injuries could be due to an increase in sport participation overall, they may also be linked to an increase in female participation in sports. Since the passing of Title IX that gave equal access to federally funded athletic programs at the high school and collegiate level for both sexes, female participation in sports as well as female injury incidence has increased.[77] Female athletes who play basketball, soccer, and volleyball are more likely to sustain an ACL injury when compared to their male counterparts, with studies reporting that female athletes have up to an 8 times greater ACL injury rate than males.[57,80] Both intrinsic and extrinsic factors account for greater injury risk in female athletes. Intrinsic factors that predispose women to sustaining an ACL injury are increased estrogen and progesterone hormone levels[73,80,89], narrower intercondylar notch[78], increased joint laxity[7, 50,73,80], and decreased neuromuscular control [73]. Muscle strength[7, 73] and the alteration of muscle control strategies[7,73,80] account for extrinsic factors of ACL injury.

During landings, there is an increased amount of force exerted on the athlete in response to the force being exerted on the ground by the athlete, known as the ground reaction force (GRF). This increase in force on the body requires the components of the lower limbs, such as the muscles, tendons, ligaments, and bone, to resist that force to prevent injuries.[27] To properly absorb the force that is placed on the lower limbs during landings, the eccentric muscles that cross the hip, knee, and ankle joints must be activated.[69] Eccentric lower extremity muscle contractions control joint motion and absorb the kinetic energy being placed on the lower limbs by decelerating the body as it meets the ground.[69] ACL injuries commonly occur during this deceleration phase of the landing, generally known as the impact phase.[61,69] An abnormal or inconsistent landing strategy that occurs during the impact phase can create a more erect landing where hip and knee joint flexion is decreased, increasing the amount of anterior shear force on those joints.[61,69,79,18,90,92]

Game-like tasks that represent conditions in which an athlete may sustain an ACL injury, such as during fatigued conditions or noncontact strategies, can create altered knee joint kinematics and kinetics. A common injury mechanism during sport is when the landing phase of a jump requires the athlete to decelerate in the horizontal direction.[92] This landing strategy requires the athlete to increase the stability of the knee joint to resist the anterior translation of the ACL that occurs from the forces acting in the horizontal direction. The stop-jump (SJ) is a task that has been used to produce this horizontal deceleration and load the ACL.[92,2] Sex-specific differences in kinematics of the SJ during the braking period of the landing have been found, with females adopting a more decreased knee[21,12] and hip maximum flexion angle and a decreased peak GRF.[34,12,21] Teater found that when an overhead target was used during the SJ, there were significant sex differences in limb stiffness of the dominant limb of the jump.[2] This further indicates that sex-specific differences occur during game-like tasks, as well as the possible heightening of limb asymmetry in landing strategies during the impact phase.

Between-limb asymmetries are a common way to predict injuries that occur in the lower extremity and to assess functional performance for when an athlete should return to sport after rehabilitation.[87,81] Inter-limb asymmetries that occur in the lower extremity create unequal force distribution, causing one limb to withstand more force than the other.[5,53] This increased force can overload the limb, being a potential factor that increases ACL loading and could lead to higher injury risk. Larger inter-limb asymmetries are related to a decrease in physical performance and an increase in non-contact injury risk.[84,81,56] Asymmetrical movement patterns can occur in athletes that perform sport-specific tasks, such as kicking a soccer ball, and can create muscle growth that is not evenly distributed among limbs.[81,82,53,54] Consistently overloading one side of the body, or the lack of equal training load distribution, can exasperate these asymmetries, creating injury risk to both the dominant and nondominant limb, with the dominant limb sustaining repetitive overloading of the muscle-tendon unit, while the non-dominant limb may grow weaker and no longer be able to sustain normal loads.[82,83,53]

Non-contact injuries often occur at a larger incidence later in a competitive match and later in the sports season, indicating that exercise-induced fatigue plays a role in this injury rate.[3,18, 72] Neuromuscular fatigue is the reduction of the amount of maximum force that can be generated by a muscle or muscle group due to exercise.[38,46,90] Neuromuscular fatigue alters physiological processes, decreasing motor control strategies such as strength[20,38], joint stability[12,38], movement coordination[38], and proprioception[12,38], which can heighten asymmetries between the limbs. The stretch-shortening cycle (SSC) is a muscle function that naturally occurs in human locomotion and exercise and provides a model to induce fatigue that would be consistent with competition situations.[86] This function occurs when the body is subject to an impact, causing the lower limb muscles to lengthen eccentrically, followed by a shortening of the muscles concentrically.[39,] Fatigue that is induced by exercises including the SSC causes disruptions to the activation of the stretch-reflex that occurs during athletic tasks, providing a negative alteration to the movement patterns.[20] The eccentric muscle contractions that are required to control joint motion and absorb the kinetic energy being placed on the body are impaired, decreasing the amount of motor control within the muscles needed to absorb the forces being placed on them, leading to increased injury risk.[86,38] It has been found that sex-specific kinematic and kinetic differences also occur with the onset of fatigue during the SJ.[7,12] While both males and females were found to have decreased knee flexion with the onset of fatigue, consistent with an increase in injury risk, females adopted a more significant decrease in knee flexion and had a larger peak proximal tibial anterior shear force than males.[7,12,21,18]

Limb stiffness is a metric that has been used to calculate how the lower extremity attenuates the GRF that is being loaded to the joints during athletic movements such as running, hopping, and jumping [64,65,67,66,94,95] and is known as the body's resistance to deformation in response to an external applied GRF. [27,47] Limb stiffness acts as a spring-mass model, providing an understanding of the way that the muscles, ligaments, and tendons exchange energy in the lower limbs, only holding true if the "material", or the mechanisms within the lower extremities, do not exceed its elastic limit.[54] The specific elastic limit of the lower extremity mechanisms is unknown, as larger limb stiffness is associated with increased injury risk to bony structures [47], reduced joint motion [27], and increased shock being placed on the body [27] while smaller limb stiffness is associated with increased soft tissue injury [47] and excessive joint motion [27]. Since the "optimal" amount of limb stiffness for a given situation or individual has not been determined, the relationship between limb stiffness and injury is not well understood. It is known, however, that having too large or too small limb stiffness can create injury to the lower extremity while, in contrast, increased limb stiffness is related to increased athletic output during sport.[70] While limb stiffness is a measure that has been used in previous research to understand mechanics during athletic tasks, it has not been heavily investigated as a metric to predict lower extremity injuries based on landing techniques used in movements. Limb stiffness asymmetry has been studied in unilateral tasks but has not been heavily evaluated during bilateral landing tasks to identify differences while the limbs are working together during an athletic movement.[54] Because of this, there is not a value for limb stiffness asymmetry that is deemed "healthy" between dominant and nondominant limbs during bilateral landings,

Although extensive research has been done to assess the biomechanics of landings that put an athlete at risk of an ACL injury, females continue to sustain ACL injuries at a disproportionate rate compared to their male counterparts. While these differences in kinetics and kinematics already exist in healthy, typical movements between males and females, it is thought that these differences will increase within athletic tasks that are more game-like and produce a landing pattern that is more common in ACL injuries. The use of limb stiffness and limb stiffness asymmetry as metrics to understand the mechanics used during landings that are predictors of lower extremity and ACL injury have not been heavily investigated.

Chapter 2: The Effect of Sex and Stretch Shortening Cycle Fatigue during the Stop-Jump on Limb Stiffness and Limb Stiffness Asymmetry

Abstract

The purpose of this study was to assess the impact of sex and fatigue on limb stiffness and limb stiffness asymmetry metrics during the stop-jump (SJ). We wanted to determine if the onset of fatigue affected the way that the body attenuated forces that occur during landing tasks that are consistent with Anterior Cruciate Ligament (ACL) injury. Thirty-four active participants (17 male, 17 female) between the ages of 18-30 years old completed SJs before and after induced fatigue. Kinetic and kinematic data was collected by force plates (AMTI, Watertown, MA, USA) and a 10-camera 3-dimentional motion capture system (Qualisys, Gothenburg, Sweden), respectively. Our first hypothesis stated that there would be a significant interaction between sex and fatigue, with females experiencing a larger dominant limb stiffness and larger limb stiffness asymmetry during the post-fatigue SJ. A linear mixed-effects model was used to identify significant sex-by-condition interactions ($p<0.05$). No significant interactions were found, but a main effect of sex was found for nondominant limb stiffness. Our second hypothesis stated that there would be significant interactions between sex and condition, with females experiencing an increase in dominant rGRF and rGRF asymmetry. A significant interaction was found for nondominant change in limb length. The main effect of condition was found for dominant rGRF and nondominant rGRF, with a main effect of sex being found for nondominant limb stiffness. No significant interactions were found for asymmetry values of limb stiffness or rGRF. Our results suggest that sex and fatigue are found to affect limb stiffness measures and its components, respectively.

Introduction

Females are known to be at greater-risk of sustaining Anterior Cruciate Ligament (ACL) injuries when participating in the same sport as males.[16,75,73,12] It is thought that possible sources of this increased injury incidence correspond to biomechanical patterns that occur during the beginning of the landing phase during an athletic task, and are associated with decreased impact attenuation and increased loading of the lower limbs.[7] Most lower extremity injuries occur during the landing phase of a movement when there is increased force that is exerted on the body, causing a resistance to this force to be produced by the body.[8] When an abnormal landing strategy is used by an athlete, it changes the way that the body attenuates the force within the lower extremity, increasing the risk of injury. Asymmetrical landings are associated with higher injury risk, as the loads being placed on the lower limbs are unequal, leading to one limb potentially being overloaded.[53] Uneven force distribution creates an imbalance in the force attenuation necessary to absorb the impacts effectively and safely. Altered landing strategies corresponding to a decrease in energy dissipation are thought to be impacted by sex and the onset of fatigue, with females adopting movement strategies that increase ACL injury risk[108,57,12] and fatigue decreasing motor control strategies that are essential for maintaining healthy movement patterns[38,50,57].

Stabilization of the lower extremity joints occurs when the athlete can accurately gather visual and proprioceptive information to maintain adequate posture and balance to complete an athletic movement.[50] Proprioception of the knee joint allows for efficient neuromotor control and joint awareness of the dynamic muscle stabilizers that cross the joint.[73] This stabilization relies on preactivated muscle tension of the lower limbs to anticipate an increase in load that will be placed on these joints during landing. The contraction of these muscles controls joint motion and is responsible for absorbing the kinetic energy being placed on the body, being an important strategy used during running, hopping, and jumping tasks that require the stretch-shortening cycle (SSC).[69]

The nature of athletic movements and muscle function is represented by the SSC. The SSC model uses the concept that the muscles in the lower limbs withstand external forces that cause them to lengthen eccentrically and shorten concentrically.[86,20,39] This type of movement is characterized by three phases. The first two phases of the movement represent the stretching of the limb, the first being the pre-activation phase in which the muscle is prepared for ground contact, and the second being the active braking phase once the foot has come in contact with the ground. The third phase is the push-off phase during which the muscle is shortening.

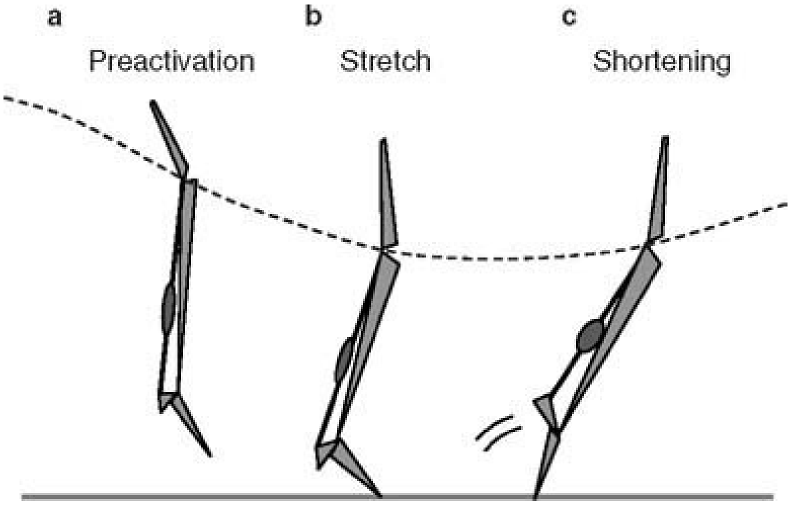


Figure 1 - Phases of the stretch-shortening cycle[86]

The voluntary muscle activation that is necessary before and during the SSC is controlled by central and peripheral neural pathways of motor control.[39] The central neural pathways and reflexes control the braking phase of the SSC, as the impact that occurs upon landing places fast loading on the limb in a short period of time. The main function of the SSC is to increase performance during the push-off, or concentric, phase of the movement by increasing the force and power output compared to isometric movements, arguably due to stored elastic energy.[39,20,86] Although this increase in performance exists because of the nature of the movement pattern, fatigue that occurs due to the SSC can disrupt the activation of the stretch-reflex due to neuromuscular impairments, decreasing muscle function and creating a loss of strength, power, and awareness of joint position.[20]

Inducing fatigue in a laboratory setting to mimic in-game fatigue has been executed in many ways. Some studies utilize a short-term protocol that uses repetitions or circuits of explosive movements[103,16,14,32,20,18] and others utilize long-term protocols that require running or sprinting intervals[103,16,32,14]. These different protocols can induce either central or peripheral fatigue in the participant. Peripheral fatigue occurs at or distal to the neuromuscular junction and is found in shorter bursts of intense exercise.[46,96] This type of fatigue originates within the muscle, decreasing the contractile strength of the muscle fibers, leading to a reduction in muscle force production.[46,96] Central fatigue originates at the central nervous system and reduces the frequency and synchronization of the firing rate of motor neurons, decreasing voluntary muscle activation strength and speed.[96] Central fatigue is found in exercise that is long in duration and typically occurs after peripheral fatigue.

Fatigue protocols commonly result in peripheral fatigue, which is localized in the muscle, and general fatigue, affecting cardiovascular and motor systems.[97] Peripheral fatigue protocols use isometric muscle contractions to induce fatigue, however, is not consistent with exercises found in sport. Therefore, a general fatigue protocol is typically used to create a fatigue state that is more consistent with competition environments as it includes exercises at submaximal activity to reduce the level of voluntary muscle activation, affecting dynamic muscle control, neuromuscular control, and lower limb movement.[97] This study will use a general fatigue protocol to induce fatigue.

Limb stiffness is a metric that assesses how the lower limbs attenuate forces being placed on the body during movement, as it defines the relationship between the deformation of the lower extremities in response to an applied force. Hooke's law is the foundation of the concept of limb stiffness, as this law states that the force needed to deform an object is directly proportional to the displacement of that object in relation to a single point mass. The equation used to calculate this is shown below, with F representing the force on the object, k representing the spring constant, and x representing the deformation that occurs on the object.

$$F = kx$$

Using this law, the force is placed on a spring, and the amount of deformation that is placed on that spring will determine how much elastic energy is stored. The stored energy will then be exerted in the opposite direction as kinetic energy. During athletic movements, the legs act as the "spring" in this model, while the body acts as the "single point mass". There are different stiffness equations that are used to model lower extremity limb stiffness. Vertical stiffness, K_{vert}, is the calculation typically used for jumping and hopping tasks confined to the vertical direction.[47] Since this calculation uses the concept that the motion occurs strictly in the vertical direction during the movement, it uses Δy in the denominator, representing the change in center of mass (COM) displacement.

$$K_{vert} = \frac{F_{max}}{\Delta y}$$

Limb stiffness, K_{limb}, is typically used to analyze the lower extremity stiffness during running and walking tasks. As the concept of K_{vert} is derived under the criteria that the body moves in one direction, that calculation is not feasible to use in movements that occur in multiple movement planes. Therefore, ΔL is used in the denominator to represent the change in limb length during the movement. The deformation found for limb stiffness represents the change in hip, knee, and ankle joints as they compress from an external force from ground contact during landing. The equation used to calculate limb stiffness is represented below.

$$K_{limb} = \frac{F_{max}}{\Delta L}$$

The stop-jump is a task that is not confined to the vertical direction, having a horizontal landing component that occurs in the first landing due to a period of running that precedes the take-off. Given the horizontal component of the stop-jump, limb stiffness better represents the movement that is being assessed in this study.

Lower limb stiffness is an integral part of athletic maneuvers and landings, as it is required for storage and re-utilization of elastic energy that is created during SSC tasks.[98] It is known that greater limb stiffness is related to increased athletic output, increasing running speed, jump height, and force output due to an increase in elastic energy that is stored in the eccentric muscle contraction that occurs at ground contact.[104] The relationship between limb stiffness and injury risk is more complex and has different outcomes, as both larger and lower levels of limb stiffness can increase injury risk. High levels of stiffness increase injury risk because of the increase in peak forces being placed on the lower limbs, while joint motion is reduced.[98] Lower levels of stiffness can, in contrast, create soft tissue injury due to increased joint motion.[98,51] These findings suggest that there is an "optimal" limb stiffness that is deemed healthy.

Limb stiffness is a measurement that needs to be more fully understood to recognize potential changes in landing kinematics or strategies that can relate to the potential lower extremity injury risk. Studies have found that, during hopping and jumping tasks, increased vertical stiffness is associated with decreased ground contact time[104,105,102], increased GRF[106,105], and increased athletic output[104]. It has also been found that limb stiffness decreases[32] and limb stiffness asymmetry increases[20, 104] with the onset of fatigue. Padua et al, however, found that, during fatigued hop testing, vertical limb stiffness did not change, but different control strategies were adopted to create the same stiffness level before and after fatigue.[107]

While past studies have assessed the kinetics and kinematics of SJ tasks, additional work is needed to create a scenario that is more consistent with the adoption of ACL injuries during competition environments. With the inclusion of more game-like movements such as a SJ with the simultaneous use of both an overhead target and the onset of fatigue, a scenario that is more representative of the development of lower limb injuries will be created. The purpose of this study was to determine differences in limb stiffness and limb stiffness asymmetry between sexes before and after neuromuscular fatigue. Prior research has demonstrated that females are more susceptible to lower extremity injury during non-contact sports compared to their male counterparts[12,16,5], it was hypothesized that females would experience a greater limb stiffness and limb stiffness asymmetry increase following fatigue than males.

Materials and Methods

Participants:

Thirty-four healthy adults (17 male and 17 female) were recruited to participate in this institutional review board (IRB#22-645) approved study. A priori power analysis was completed based on pre and post fatigue drop landing vertical stiffness asymmetry effect size from Knihs et al. 2021. A sample size of 27 (significance of 0.05, power of 0.90, η^2 of 0.102, effect size of 0.34) was determined using G*Power (Heinrich Heine University Dusseldorf, Germany). This sample size was increased to 34 (17 males and 17 females) to account for potential data collection or processing technical issues. Two participants were removed from the analysis (1 male and 1 female) due to poor data quality. To our knowledge, there has not been a study that has assessed the interactions between sex and fatigue on limb stiffness and limb stiffness asymmetry during the stop-jump.

To be enrolled, each participant had to (1) exercise at least three days a week for at least 30 minutes a day, (2) have a body mass index (BMI) of less than 35, (3) have previous or current experience in sports (i.e., high school varsity, club, intramural, and/or recreational level) that required jump landing maneuvers, and (4) had a shoe size between 7.5 and 14 if they were male and between 6 and 11 if they were female. Participants were excluded if they (1) had current self-reported lower extremity pain, (2) had had any lower extremity injury within the last two months that prevented them from completing regular physical activity, (3) had a history of serious lower extremity injury that required surgery and/or extensive physical therapy, (4) had participated in a jump landing training program, or (5) were pregnant.

Testing Procedure:

Prior to testing, all participants completed informed consent. The participant's demographic information was collected in REDCap (Research Electronic Data Capture)[112,113], a secure online database. Age (years), sex assigned at birth, race, height, weight, and lower extremity limb dominance were recorded. Lower extremity limb dominance was defined as the limb that the participant used to kick a ball that was rolled towards them. Maximum vertical jump height was recorded using the distance between the participant's standing reach height and the height of their maximum countermovement jump (CMJ). The participant was instructed to raise one hand overhead and place a piece of tape on the wall as far as they could with their full foot still on the ground. They then placed a piece of tape at the highest spot that they could reach while performing the CMJ. The distance between the standing reach and maximum vertical jump was recorded using a measuring tape. Each participant was fitted with athletic compression shorts and a standardized pair of running shoes (Nike Pegasus, Portland, OR, USA) to be used during testing.

Each participant had 27 retroreflective markers placed at anatomic landmarks of the lower-extremities in a Modified Helen-Hayes configuration (Figure 2).

Figure 2 – Modified Helen-Hayes marker set

These markers were used to determine the kinematics of each segment of the lower extremities such as the pelvis, thigh, shank, and foot. Markers were placed bilaterally on the anterior superior iliac spines, posterior superior iliac spine, iliac crest, and between the L4 and L5 vertebrae to define the pelvis segment. Markers were placed bilaterally on the medial and lateral epicondyles and malleolus, the greater trochanter, the first and fifth metatarsals, and the superior, inferior, and lateral calcaneus to define distal and proximal ends of each segment. In addition, 4-marker rigid clusters (SAM splints; SAM, Medical, Tualatin, OR, US) were placed on the thigh and shank to track the motion of these segments (16 additional markers). A static trial was collected, with the participants instructed to stand with their feet shoulder-width apart on the force plates, having one foot on each force plate with their weight distributed evenly. They were to stand in the anatomic position for 5 seconds to ensure that the cameras could collect the position of each marker to define the body segments. Once the static trial was completed, the medial epicondyle, medial malleolus, and 1st metatarsal markers of each limb were removed to allow for more freedom of movement and to decrease the risk of markers coming off during testing.

Prior to the completion of the tasks, a rubber strip was placed on the floor at 50% of the participant's height away from the end of the force plate and a suspended ball was raised to 80% of their maximum jump height. The participants were then instructed to perform seven stop-jumps (SJ)s, being told to run forward up to five steps, take off at the location of the rubber strip with one leg, land directly beneath the suspended ball with one foot on each force plate, quickly jump vertically to touch the ball with both hands, and land again on the force plates with a controlled landing (Figure 3).

Figure 3 – Stop-jump task in chronological order from left to right, top to bottom

The participants were not told which foot to initiate the SJ with for the take-off but were asked to keep it consistent between each trial. They were instructed to focus on the suspended ball for the entire duration of the SJ, once their foot was placed on the rubber strip for take-off. Participants were able to practice the SJ up to three times before the task was recorded to ensure they were comfortable with the movement. Each participant completed seven successful pre-fatigue SJs. A trial was unsuccessful if (1) either of their feet did not land fully on a single force plate, (2) they did not land under control with no foot movement on the second landing, or (3) they did not direct their attention to the suspended ball. Thirty seconds of rest were given between each SJ to ensure that fatigue would not affect the results. During each of the 7 SJs, motion capture and force plate data were simultaneously collected. Kinematic data was collected through a 10-camera capture system (Qualisys, Gothenburg, Sweden) at a sampling frequency of 240 Hz. Kinetic data was collected through an embedded force plate system (AMTI, Watertown, MA, USA) at a sampling frequency of 1200 Hz.

Once 7 pre-fatigue SJs were successfully captured, the participants were provided instructions on the series of exercises they were to complete to initiate fatigue. The fatigue protocol consisted of completing 10 continuous counter movement jumps (CMJ), an agility ladder drill, 10 repetitions of jumping lunges on each leg, and jogging back to the CMJ station. After completing a cycle of the fatigue protocol, the participants self-reported their fatigue level based on the Borg's RPE scale[111]. This scale ranges from 0-20, with a value of 10 representing light exertion, 15 representing hard, or heavy, exertion, and 20 representing maximal exertion. If the participant indicated an RPE of 14 or less, they completed another cycle of the fatigue protocol. Once the participant indicated an RPE of 15 or greater, the fatigue protocol was stopped. If an RPE of 19 or greater was indicated, they were required to rest until it became less than 19 to confirm that they were not completing the SJ at maximum exertion. If the RPE was between 15 and 19, they were instructed to immediately complete 2 repetitions of the SJ to be recorded. After two SJ trials were successfully completed, the participant was asked for their RPE again and if it remained between 15 and 19, they completed another 2 SJ trials. If their RPE was below 15, they completed the fatigue protocol until it reached 15 or greater and two more trials of the SJ were completed. This cycle continued until 7 successful post-fatigue trials were completed.

Data Analysis:

The kinematic and kinetic data was collected through Qualisys Track Manager (Qualisys AB, Gothenburg, Sweden) and each of the retroreflective markers was labeled throughout each trial. All labeled SJ trials were imported into Visual3D (HAS-Motion, Inc., Kingston, Canada) and filtered using a lowpass 4th order Butterworth filter with a cutoff frequency of 7 Hz for kinematic data and 100 Hz for kinetic data. The first landing was then identified using the Automatic Gait Events command, in which the start and end of the landing was defined by event labels for both kinematic and kinetic events. The events were identified using a threshold of 10 N indicating contact with the force plates, with the heel strikes of the landing being the initial frame where the force exceeds this threshold, and the toe-off being the first frame subsequent to a force less than this threshold. Kinetic data was normalized by body weight (%BW) to account for differences in body mass between participants. Three-dimensional time-series data of the center of pressure (COP), ground reaction force (GRF), hip joint center, pelvis segment location, as well as hip, knee, and ankle angles of both limbs were exported from Visual3D. A custom MATLAB (MathWorks, Natick, MA, USA) code was used to calculate the outcomes of interest for each trial for every participant.[2] The outcomes of interest included limb stiffness ($\underline{K_{limb}}$), the maximum resultant GRF (Fmax) and the change in limb length (ΔL) from initial contact (IC) to the point of Fmax.

The rGRF was normalized by kinetic energy (KE), shown by equation 1 where the variables of “m” and “v” represent the subject's mass and velocity, respectively. The velocity used in the KE equation was defined as the velocity of the center of mass (COM) at initial contact. The pelvis segment defined in Visual3D was used to calculate the COM, using the x, y, and z-coordinate positions. Normalization of the rGRF to KE allows for comparison between-sex and participants, as it accounts for differences in jump height and body mass.[99,2]

$$KE = \frac{1}{2} \times m \times v^2 \tag{1}$$

The normalized rGRF was then projected onto the limb length vector for each limb during the landing, where the limb length vector measures the distance between the COP and hip joint center.[2] The maximum value of this resultant GRF was determined as Fmax. ΔL was defined as the distance between the three-dimensional COP and the hip joint center from initial contact to the point of Fmax. To standardize measurements to adjust for individual variations in limb length, ΔL was divided by the limb length at IC for each participant. The limb stiffness of each limb was calculated as the division of the calculated Fmax and ΔL using equation 2.

$$K_{limb} = \frac{F_{max}}{\Delta L} \tag{2}$$

Asymmetry between limb stiffness and Fmax was computed using the Bilateral Asymmetry Index (BAI-1).[28] This equation was used to assess asymmetry, as it compares the two sides of the body to each other, not calculating each side individually. The difference between the dominant and nondominant side is divided by the sum of the two limbs and calculated for each trial of every participant.

$$BAI - 1 = \frac{D - ND}{D + ND} \times 100 \tag{3}$$

Larger values indicate larger asymmetry between limbs, with a positive value indicating the dominant limb was favored, while a negative value results from favoring the nondominant limb.

Statistics:

Limb stiffness asymmetry, GRF asymmetry along with dominant and nondominant limb stiffness, rGRF, and the change in limb length were calculated for each trial of every participant. These metrics were output for pre-fatigue and post-fatigue conditions and reported by group as the male and female means and standard deviations for each task. The outcomes were analyzed for outliers by determining if the values were greater than two standard deviations from the mean based on group (i.e., male, female) and testing condition (i.e., pre-fatigue, post-fatigue). If outliers were identified, they were removed from the sample.

Linear mixed effects models were performed in R Studio (RStudio, Boston, Massachusetts) to compare measures between sex and across condition (pre-fatigue, post-fatigue). The main effects of the model were sex and fatigue condition, with the random effects being each participant. The dependent variables in this model were dominant and nondominant limb stiffness, dominant and nondominant GRF, dominant and nondominant change in limb length, and the asymmetry of limb stiffness and GRF. This model was used to find sex-by-condition interactions for each dependent measure, with a significance level of $p<0.05$ being used to determine statistically significant differences. If a significant interaction was found between sex and fatigue condition for the measure ($p<0.05$), Tukey's Honestly Significant Difference post-hoc analyses were performed to determine the significance of each pairwise comparisons between sex and condition. If no interaction was identified as significant, then main effects of sex and fatigue condition were calculated.

Results

A significant sex-by-condition interaction was found for the nondominant change in limb length ($p=0.005$), however there were no significant pairwise outcomes from the post-hoc analysis ($p>0.05$) (Table 1). No significant differences were found for dominant and nondominant limb stiffness, dominant and nondominant rGRF, dominant change in limb length, limb stiffness asymmetry, and rGRF asymmetry (Table 1). Women demonstrated a smaller change in limb length during landing when fatigued, while men demonstrated a larger change in limb length. (Figure 4). A significant main effect of sex was found for nondominant limb stiffness (p=0.037), dominant limb GRF (p=0.047), and nondominant limb GRF (p=0.007). Table 2 indicates main effect interactions for nondominant limb stiffness, dominant and nondominant GRF, and nondominant change in limb length values. Table 3 indicates the mean and standard deviation of each outcome measure.

Table 1 - Sex:Condition interactions for dominant and nondominant K_{limb}, rGRF, and change in limb length, K_{limb} asymmetry, and rGRF asymmetry; ***Bold**** *indicates a significant interaction*

	Sex:Condition
Dominant K_{limb}	0.416
Nondominant K_{limb}	0.481
Dominant rGRF	0.319
Nondominant rGRF	0.540
Dominant ΔL	0.380
Nondominant ΔL	**0.005***
K_{limb} Asymmetry	0.428
rGRF Asymmetry	0.081

Table 2 - Main effect interactions of sex and condition for nondominant K_{limb}, dominant Fmax, and nondominant Fmax; ***Bold**** *indicates a significant interaction*

	Sex	Condition
Nondominant K_{limb}	**0.038***	0.912
Dominant rGRF	0.095	**0.047***
Nondominant rGRF	0.560	**0.007***

Table 3 - Mean and standard deviations for each sex before and after fatigue for dominant and nondominant K_{limb}*, rGRF, and change in limb length, K_{limb} asymmetry, absolute value K_{limb} asymmetry, and rGRF asymmetry;* ***Bold**** *indicate significant interactions,* ***Bold+*** *indicate main effect of sex, and* ***Bold%*** *indicate main effect of condition*

	Pre-Fatigue Mean ± SD		Post-Fatigue Mean ± SD	
	Male	Female	Male	Female
Dominant K_{limb} (%BW/m)	14.9±3.40	12.5±3.38	16.0±3.39	13.7±3.39
Nondominant K_{limb} (%BW/m) +	17.14±3.11	7.94±3.09	16.95±3.1	7.75±3.31
Dominant rGRF (%BW) %	0.56±0.02	0.62±0.02	0.54±0.02	0.60±0.03
Nondominant rGRF (%BW) %	0.54±0.03	0.57±0.03	0.51±0.03	0.54±0.03
Dominant ΔL	0.12±0.01	0.11±0.01	0.11±0.01	0.1±0.01

Nondominant ΔL * %	0.10±0.02	0.13±0.02	0.12±0.02	0.12±0.02
K_{limb} *Asymmetry (%)*	-2.62±5.12	11.45±5.04	3.67±5.08	13.31±5.05
Abs K_{limb} *Asymmetry (%)*	31.1±3.05	27.0±3.02	30.9±3.04	26.8±3.02
rGRF Asymmetry (%)	2.35±1.83	5.07±1.82	3.85±1.82	6.08±1.82

Figure 4 shows the interaction plot for significant outcomes in nondominant limb change in limb length. Figures 5-7 show plots of significant main effects of conditoin for outcomes in nondominant limb stiffness, dominant limb rGRF, and nondominant limb rGRF, respectively.

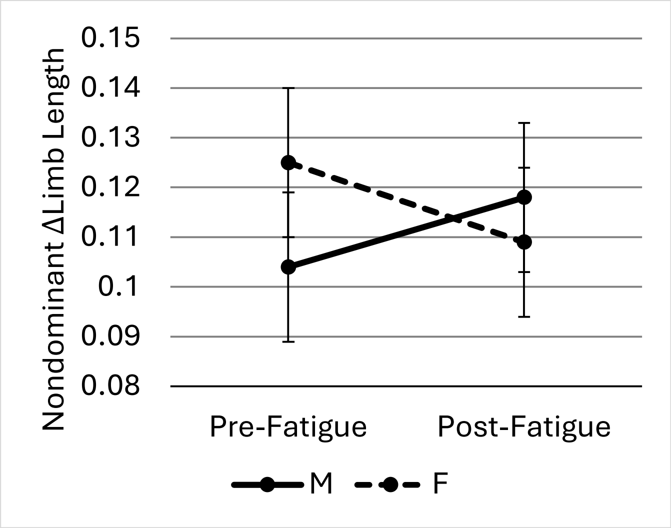
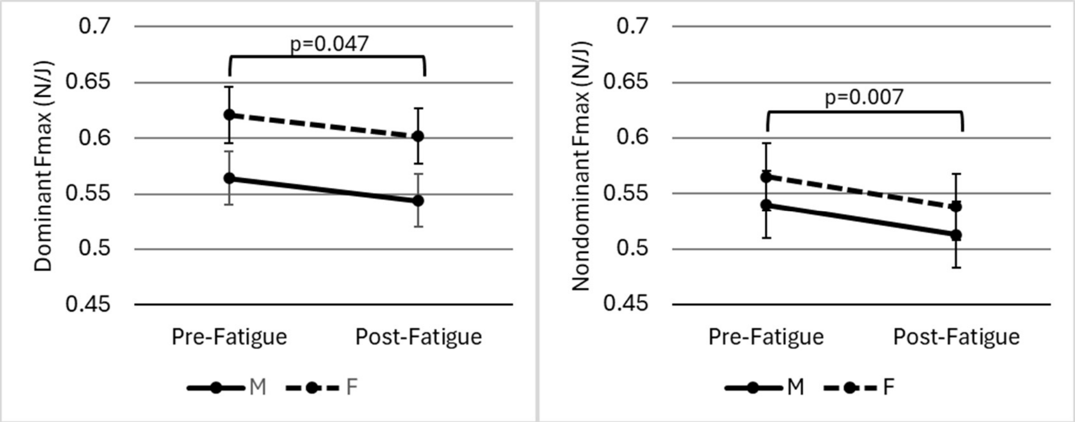


Figure 4 - Interaction plot of nondominant change in limb length for male and female between conditions



Figures 5-6 – Main effect plots of dominant and nondominant rGRF (Fmax) for male and female between conditions, indicating significant differences between condition

Discussion:

This study aimed to investigate the effect of sex and fatigue on limb stiffness and limb stiffness asymmetries during a stop-jump task with an overhead target to simulate a game-like task. As females are known to sustain ACL injuries at a greater rate than males, it was hypothesized that females would adopt different landing strategies than males, particularly strategies that are associated with ACL injuries, after fatigue was induced. Our hypothesis was that there would be a significant interaction between sex and fatigue, with females experiencing greater dominant limb stiffness and a greater limb stiffness asymmetry during the post-fatigue SJ. Our second hypothesis was that there would be a significant interaction between sex and fatigue with females experiencing greater dominant rGRF and rGRF asymmetry. Our findings did not support the first hypothesis, however, they did support the second hypothesis.

The only significant sex-by-condition interaction was found for nondominant change in limb length (p=0.005), with no significant interactions for limb stiffness (p=0.481) or limb stiffness asymmetry (p=0.428) (Table 3). The change in limb length values were normalized to limb length at initial contact, so the difference in limb length that exists between-sex and between participants should be accounted for. This change in limb length showed males experiencing a smaller value than females before fatigue (M=0.104 ± 0.015 m, F=0.125 ± 0.015 m), but a larger value post-fatigue (M=0.118 ± 0.015 m, F=0.109 ± 0.015 m).

Although a significant main effect of sex was found for nondominant limb stiffness, our hypothesis was not supported, as we had predicted that females would experience a larger limb stiffness value than males. These findings, however, show that fatigue did not significantly affect limb stiffness measures, as the results were similar across sex for both males and females before (M=17.14±3.11, F=7.94±3.09) and after (M=16.95±3.1, F=7.75±3.1) fatigue (Table 3), but were significantly different across sex. A study done by Teater, et al found that females experienced a smaller limb stiffness in the SJ compared to males, consistent with what was found in this study, however fatigue was not considered.[2] Limb stiffness calculated during a 30-s vertical jump test showed that vertical stiffness decreased throughout the test, suggesting that fatigue plays a role in the decrease of limb stiffness.[102] In contrast, however, literature has also found that a more erect landing strategy is adopted in the SJ[12], single leg landing tasks[101,14], and cutting[16] tasks after fatigue. This erect landing strategy exhibits decreased attenuation of forces and increased limb stiffness. While literature has diverse and sometimes contrasting findings for the effect of fatigue on limb stiffness measures, our findings suggest that only differences between sex exist for limb stiffness values alone.

There was a significant main effect for fatigue condition on the rGRF for both the dominant and nondominant limbs, with the rGRF decreasing for both males and females from pre to post fatigue (Figures 6-7). As our hypothesis stated there would be a significant increase in GRF from pre to post fatigue, our findings did not support our hypothesis. This significant decrease in the rGRF in the post fatigue conditions may be a result of a decrease in the ability to produce force in the lower extremity during the SJ task. Fatigue that occurs from the stretch-shortening cycle (SSC) decreases the ability of the lower limb muscles to produce force during the take-off of the jump, resulting in a smaller GRF being placed on the ground during the first landing of the SJ compared to pre-fatigued conditions. Another potential reason for this decrease in the rGRF could be a result from different landing mechanics strategies that are adopted to compensate for this fatigued condition. An increase in knee flexion angle could be a strategy that would attenuate the force placed on the body, decreasing the rGRF. Further results of this study found that maximum knee flexion angle increased with the onset of fatigue in both males and females, providing evidence to support this reasoning.

Based on literature, it has been observed that females land from a SJ with greater rGRF compared to males with and without the use of an external target[2]. As rGRF is made up of forces in the vertical, anterior-posterior, and mediolateral direction, findings of another study observed an increase in vertical ground reaction force (vGRF) and posterior ground reaction force (pGRF) with the onset of fatigue in female athletes performing forward drop vertical jumps, not consistent with the findings of this study.[11] Although vGRF and pGRF were the metrics found in existing literature, these two directional components make up the calculation of rGRF, assuming an increase in both components would result in an increased rGRF, allowing these values to be comparable. These differences in findings compared to our results may occur due to the difference in assessing rGRF and the combination of vGRF and pGRF, as the rGRF takes into consideration the three-dimensional components, not just the vertical and posterior component of the force. This observed increase of vGRF and pGRF in literature also may be due to the nature of the task, as it was assessing a drop landing, not a jumping task that has a running component preceding the landing. As the SJ uses a horizontal component and is a bilateral landing task, it makes sense that our findings are similar to that of the study using assessments of rGRF in the SJ task.

This study was able to identify differences between sex and condition for limb stiffness and its components, but limitations exist that can be improved on for future studies. This study used a general fatigue protocol to induce fatigue, affecting cardiovascular and motor systems of the participant. Borg's RPE scale was used to assess the degree of fatigue that the participant felt, which is highly dependent on the participants' perception of fatigue. While there was not any diagnostic data collected to monitor fatigue levels such as heart rate, fatigue was subjective and assessed at the participant's discretion. Mental fatigue could have also played a negative role in the participant's indication to their fatigue level, as it is possible that the participant experienced a decrease in commitment to continue the fatigue protocol because it was challenging. While fatigue may have been variable between each participant, computing the coefficient of variation indicated that there was not a significant difference in the trial-by-trial variability of limb stiffness from pre-fatigue to post-fatigue SJ tasks. This suggests that there is not a learning effect that needs to be considered between the two conditions. Another limitation is that while this study aimed to create a more game-like task by including an overhead target and the induction of fatigue, additional demands could be added such as unanticipated movements.

The effect of sex and fatigue on limb stiffness and limb stiffness asymmetry had not been investigated prior to this study. The only significant sex-specific difference in limb stiffness measures occurred in the nondominant limb stiffness, indicating that there are not many sex-specific differences that occur with the onset of fatigue. Condition-specific differences occurred pre- to post- fatigue for dominant and nondominant GRF, indicating that fatigue plays a role in kinetic differences for both males and females in the SJ.

Overall, significant main effects were found for nondominant limb stiffness, nondominant change in limb length, and both dominant and nondominant rGRF values. A lack of interactions for the main outcomes of this study could be due to variance in participant's landing strategies that are adopted after fatigue, as the outcome measures had large standard deviations, indicating large variation within the data (Table 3). Another reason for a lack of significant interactions could be that there was not a large enough effect size for the outcome measures. After running a post-hoc power analysis on dominant limb stiffness values, an effect size of 0.375 and a power of 0.335 was found, indicating that there is not a strong relationship between sex and limb stiffness. These outcomes show that a larger sample size is needed to find if a significant interaction exists. Our results suggest that sex and fatigue are found to affect limb stiffness measures and its components, respectively.

Chapter 3: The Impact of Sex and Fatigue on Asymmetrical Lower Limb Mechanics During the Stop-Jump

Abstract

The purpose of this study was to assess the impact of sex and fatigue on asymmetries of peak knee flexion angle, knee flexion angle at initial contact (IC), and loading rate. Kinetic and kinematic data was collected for healthy, active participants throughout stop-jump (SJ) tasks, before and after induced fatigue, using force plates (AMTI, Watertown, MA, USA) and a 3-dimensional motion camera system (Qualisys, Gothenburg Sweden), respectively. Our hypothesis stated that there would be significant interactions between sex and condition with females experiencing larger asymmetry values in the post-fatigue SJ. Our hypothesis was partially supported, with significant interactions occurring for peak knee flexion angle asymmetry (p=0.008) and absolute value knee flexion angle at IC asymmetry (p=0.009). Post-hoc analysis for both knee flexion asymmetries indicated significant interactions in females from pre- to post- fatigue task condition. Loading rate asymmetry did not show any interactions or main effect differences. These findings suggest that females adopt a more asymmetrical landing pattern after induced fatigue, creating a landing pattern that increases their risk of sustaining lower extremity injuries compared to males.

Introduction

It has been well-established that females are at a greater risk of lower extremity injuries than males, as they experience larger rates of ACL ruptures each year.[5,41,49] Most ACL injuries occur from non-contact landings in sports that involve movements requiring sudden stops and large decelerations such as cutting, pivoting, or jump landing tasks.[12,49,16] ACL injuries also tend to occur at larger rates later in athletic competitions, suggesting that fatigue may be a contributing factor to the occurrence of lower extremity injuries.[72] While research has identified anatomical and biomechanical differences between sexes[21], the exact reason for the larger incidence of ACL ruptures in females remains unclear. By understanding the biomechanics that are involved in athletic movements such as landing during a fatigued game-like condition, we can better identify factors that cause lower extremity injuries in female athletes.

During landing tasks, the body relies on the use of motor control[50,38,12,73], proprioception[38,93,73], joint stabilization[38,93,73], and joint coordination[20,5] to effectively dissipate and attenuate the forces that are being placed on the body during the task. Landing patterns that allow for this attenuation of forces, such as landing that uses a more flexed posture, decrease the risk of injury to the lower extremity.[32,85] A more flexed landing position where the lower extremity joint angles are increased can absorb the forces being placed on the joints by distributing the load on the body over a longer period of time.[19]

It has been found that landing preparations are preprogrammed before impact and play a role in the motion patterns that occur upon ground contact.[7] The preparation for these landings has been found to have sex-specific differences, with females experiencing a more erect, upright position during the initial contact of landings.[34] The eccentric action of pre-stretch of the muscles and tendons in the lower limb initiate the storage of elastic energy, which is the initial stage of the stretch-shortening cycle (SSC).[87] The second phase continues this stretch, or lengthening, of the muscles once the lower limbs contact the ground, requiring deceleration of the body by these muscles. Fatigue affects the motor control necessary to efficiently utilize the SSC, decreasing the force attenuation during the lengthening phase and force production of the shortening phase. As motor control regulates the way that the body preactivates the leg extensor muscles during these landings, it is thought that fatigue induced by SSC exercises will impact the motion patterns and landing mechanics during a SJ task, creating a landing strategy that is more consistent with injury.

As the landing mechanics at initial contact and during the period of ground contact are important to assess potential injury mechanisms, the loading rate, maximum knee flexion angle, and knee flexion angle at IC will be analyzed in this study. Previous literature suggests that increased knee flexion angle during the active landing and at the point of initial contact can aid in reducing the load placed on the ACL during these landings.[7] At IC, females have been found to demonstrate a smaller knee flexion angle than males in the SJ task with no fatigue[34,2] and adopt a decreased knee flexion angle after fatigue compared to pre-fatigued conditions[19,16]. While these studies assessed knee flexion at IC, there has not been a comparison between sexes before and after fatigue for this metric. Females also have been found to have a smaller maximum knee flexion angle during the active landing of the SJ compared to males[12,34,21], with Chappell et al finding that knee flexion angle decreases with the onset of fatigue for both sexes.[12] While

Chappell et al. compared males and females in the stop-jump task before and after fatigue, the sample size in the study was smaller than the current study and did not have a specific way of indicating fatigue, as it was based on “a state of volitional exhaustion”.[12] In studies focusing on only female athletes, it was found that knee flexion angle decreased with fatigue compared to non-fatigued conditions in the stop-jump.[19.18] Loading rate, in contrast, has not been heavily reported in literature assessing jump landings and has resulted in inconsistent findings. Some studies that focused on male populations have found that fatigue does not have a significant effect on loading rate in drop landings[110], while others have observed that loading rates increase after fatigue[109]. A study by Bell et al. found that in drop landings males and females had similar loading rates before fatigue, with males experiencing a faster loading rate than females after fatigue.[103] Loading rate, however, has previously not been assessed in the stop-jump task across sexes before and after the induction of fatigue.

Although the findings associated with all of these metrics are inconsistent in the literature, a growing body of evidence suggests that females create landing patterns that place a larger load on the ACL than males do, as they land with a more erect position. Further research, however, is necessary to gain a better understanding of the differences in landing mechanics between sexes after fatigue, particularly by assessing asymmetries between limbs. This study will introduce the use of asymmetry metrics between limbs for maximum knee flexion angle, knee flexion angle at IC, and loading rate to further understand sex-specific injury predisposition during pre- and post-fatigue landings.

Thus, the purpose of this study is to examine the influence of fatigue and sex on lower extremity landing mechanics to gain a further understanding of strategies used during sport-specific tasks. The use of the SSC to induce fatigue provides a model that is consistent with fatiguing muscles associated with athletic movements found in game-like scenarios. We hypothesize that there will be a significant interaction between sex and fatigue with females experiencing larger asymmetry value for loading rate, peak knee flexion, and knee flexion at IC after the induction of fatigue in the SJ with an overhead target when compared with males.

Materials and Methods

Participants:

Thirty-four active adults between the ages of 18 and 30 were recruited to participate in this study (IRB#22-645), with two participants being excluded due to low-quality data. Inclusion criteria required each participant to: (1) have previous sport experience that included jump landing tasks (i.e., high school varsity, intramural, recreational, and/or club level), (2) exercise for a 30-minute duration at least three times a week, (3) have a body mass index under 35%, and (4) wear between a size 7.5-14 or 6-11 shoe if they were male or female, respectively. Exclusion criteria for participation were: (1) current lower extremity pain, (2) physical inactivity due to a lower extremity injury that had been sustained in the last two months, (3) a history of surgery or physical therapy due to a lower extremity injury, (4) prior instruction on proper landing mechanics from a jump landing program or (5) current pregnancy.

Testing Procedure:

Each participant provided informed consent prior to testing by signing a digital form that was recorded in a secure web application database, REDCap (Research

Electronic Data Capture)[112,113]. Demographic information including the participant's height, weight, sex assigned at birth, age, race, and lower extremity dominance were also collected in this database. Lower extremity dominance was established by rolling a ball to the participants and asking them to lightly kick the ball back. The limb that the participant used to kick the ball with was determined as the dominant limb. Participants were provided with athletic attire to wear during testing, including fitted compression shorts and a standardized pair of running shoes (Nike Pegasus, Portland, OR, USA). To quantify maximum vertical jump height, the difference between the participant's standing reach height and jumping height during a counter movement jump (CMJ) was recorded. The participant was instructed to stand facing the wall and place a piece of tape where their arm could reach the furthest without their feet coming off the floor. They then completed 3 vertical jumps, with the highest jump being used to record the difference between the two heights using a measuring tape. A rubber strip was placed at 50% of the participants height away from the end of the force plates and a suspended ball above the first two force plates was placed at 80% of their maximum vertical jump height.

Each participant was fit with 27 retroreflective markers that were placed on anatomical landmarks of the lower extremity in a modified Helen-Hayes configuration to create limb segments (Figure 1).

Figure 1 - Modified Helen-Hayes marker set

Markers placed on the anterior and posterior superior iliac spine, iliac crest, and the sacrum, defined between the L4 and L5 vertebrae, established the pelvis segment. Markers placed at the medial and lateral epicondyles were used to define the distal end of the thigh segment and the proximal end of the shank segment, with the medial and lateral malleolus markers used to define the distal end of the shank segment. Markers placed at the first and fifth metatarsals and at the superior, inferior, and lateral calcaneus were used respectively to define the foot segment distally and proximally. Rigid 4-marker clusters (SAM splints; SAM, Medical, Tualatin, OR, US) were placed on the lateral side of the participant's shank and thigh to aid as references to the single anatomical markers to accurately track dynamic movement of each segment. Once all the markers were placed on the participants, a static trial was collected, instructing the participant to stand on the first two embedded force plates with their feet shoulder width apart in the anatomic position, evenly distributing their weight on each limb. The trial was collected for 5 seconds in order for the cameras to track the placement of each of the markers in relation to each other to define the body segments once dynamic movement occurs. Due to the potential of falling off during the tasks, the medial epicondyle and malleolus and the first metatarsal markers were removed from the participant’s body before the start of data collection.

To complete the stop-jump (SJ) tasks, the participants were asked to take up to 5 steps before taking off of the rubber strip placed on the floor with one foot, land bilaterally on the force plates directly beneath the suspended target, immediately jump vertically as high as they could to reach the target, and land back on the force plates with control (Figure 2).

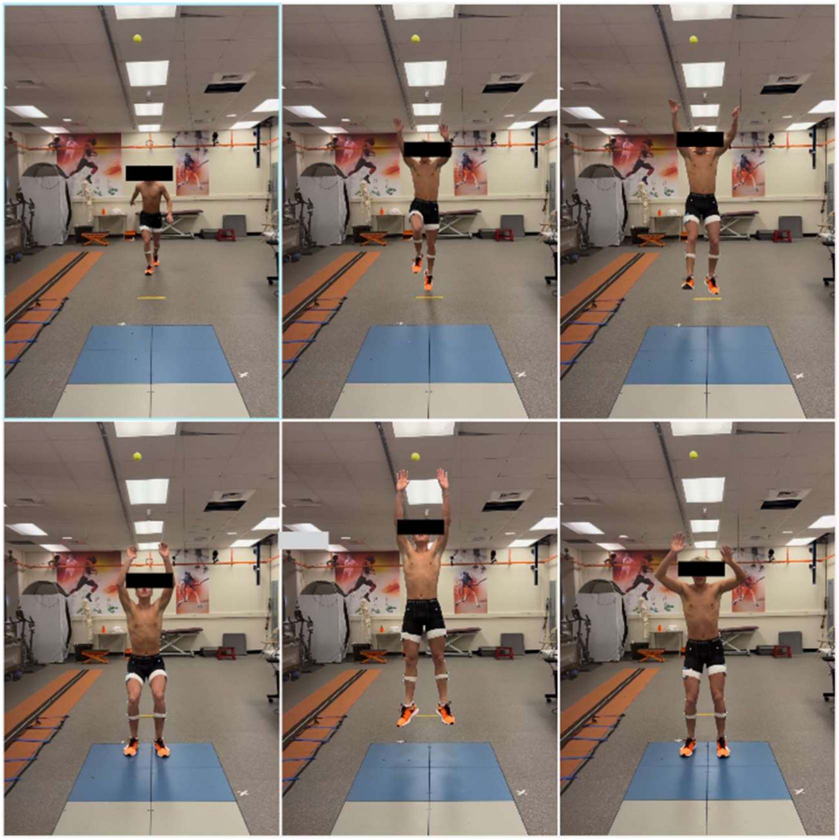


Figure 2 - Chronological order of stop jump task from left to right, top to bottom

It was ensured that each participant took off of the rubber strip with the same foot for each trial. After takeoff, their attention was to be directed to the suspended target above the force plate until the end of the trial. A maximum of 3 practice trials were permitted for participants to familiarize themselves with the task before seven successful SJs were recorded. Trials were deemed successful and could be used only if the following criteria were met: both feet landed fully on each force plate, the second landing was completed with control, and the participant's attention was directed to the ball after take-off.

Once the pre-fatigue SJs were completed, the fatigue protocol was explained to the participants. The circuit included the following; 10 continuous countermovement jumps (CMJ), a footwork drill across an agility ladder, 20 total jumping lunges, and a jog to the start of the circuit. The rate of perceived exertion (RPE) of each participant was self-reported after completion of the circuit. This RPE was based on the Borg rating of perceived exertion scale[111], ranging from values of 0 to 20, with 0 indicating resting state and 20 indicating maximal exertion. An RPE between 15-19, indicating heavy, but not maximal exertion, was to be achieved by each participant before completing the post-fatigue SJs. To ensure this level of fatigue, if an RPE below 15 was reported, the participant was asked to complete another round of the circuit and re-report their fatigue level. If their RPE was between 15-19, the circuit would not be initiated again, and 2 successful SJs would be completed. Immediately after the SJs, the RPE is reported again, and if the fatigue level was still within the desired range, another 2 SJs would be completed. If the fatigue level was below 15, the fatigue circuit was initiated again. If the RPE ever reached a level of 20, SJs were not completed until it lowered to a 19. This process was repeated until seven successful SJs were recorded.

Data Analysis:

Qualysis Track Manager (Qualysis AB, Gothenburg, Sweden) was used to collect kinematic data from each retroreflective marker and kinetic data from the force plates throughout each SJ trial. Visual3D (HAS-Motion, Inc., Kingston, Canada) was used to import each labeled trial, normalizing kinetic data by body weight (%BW) and using a 4th order Butterworth filter to filter the data with a cutoff frequency of 7 Hz for 3D motion camera kinematic data and 100 Hz for force plate kinetic data. The first landing of the SJ was then identified using an automatic force detection system within Visual3D that used a 10 N threshold that corresponded to ground contact on the force plates. Three-dimensional center of pressure (COP), pelvis segment, hip joint center, and the angles of the hip, knee, and ankle of both limbs were exported using an export pipeline. The maximum knee flexion angle, knee flexion angle at IC, and loading rate (LR) of each limb for every participant during the first landing of the SJ was calculated using MATLAB (MathWorks, Natick, MA, USA) code. LR was calculated as the ratio of the peak vertical ground reaction force (vGRF) and time from IC to the point of peak vGRF. This was calculated using equation 1 below.

$$LR = \frac{vGRF_{max}}{time} \tag{1}$$

The inter-limb asymmetry of maximum knee flexion, knee flexion at IC, and LR was calculated using the Bilateral Asymmetry Index (BAI-1) in equation 2 for every trial of each participant. This index is used to calculate the percent asymmetry between limbs during bilateral landing tasks where larger magnitude of the resultant value indicates a larger amount of asymmetry between limbs. Positive outcome values indicate a favor of the dominant (D) limb and negative outcome values indicate a favor of the nondominant (ND) limb.

$$BAI - 1 = \frac{D - ND}{D + ND} \times 100(\%) \tag{2}$$

Statistics:

Asymmetry values for loading rate, maximum knee flexion, and knee flexion at IC were calculated between limbs for each trial, both before and after fatigue, using the BAI-1. These values were calculated for every participant and reported as the mean and standard deviation for each sex for both conditions. Outliers were measured as being two standard deviations away from the mean for a specific sex and condition, being excluded from the data if they fit this criterion.

Linear mixed effect models were run in RStudio (RStudio, Boston, Massachusetts) between sex and across condition (pre-fatigue, post-fatigue) for each outcome variable. The fixed effects for this model were sex and condition, comparing male and female groups across the pre- and post- fatigue tasks, with the random effect being each participant. The dependent variables were maximum knee flexion angle asymmetry, knee flexion angle at IC asymmetry, and loading rate asymmetry.

Interactions were considered significant if the significance level was found to be p<0.05. If a significance level within this range was found between sex and condition for any measure, a post-hoc analysis was performed using Tukey's Honestly Significant Difference to indicate pairwise comparisons between sex and condition that were significant. If significance was not found between any given pairwise comparison, the main effects were tested independently.

Results

Significant interactions were found between sex and condition for maximum knee flexion angle asymmetry (p=0.008) and the absolute value knee angle asymmetry at initial contact (p=0.009). Post-hoc analysis showed significant differences from pre- to post-fatigue in females for maximum knee flexion angle asymmetry (p=0.005) and absolute value knee angle asymmetry at IC (p<0.001). No significant interactions (p=0.107) or main effects of sex (p=0.245) or fatigue condition (p=0.867) interactions were found for loading rate asymmetry.

Table 1 – Sex:Condition interactions for maximum knee flexion angle asymmetry, absolute value knee flexion angle at IC, and loading rate asymmetry; ***Bold**** *indicates significant interactions*

	Sex:Condition
Maximum Knee Flexion Angle Asymmetry	**0.008****
Abs Value Knee Flexion Angle at IC Asymmetry	**0.009****
Loading Rate Asymmetry	0.107

Table 2 - Mean and standard deviations for each sex before and after fatigue for maximum knee flexion angle asymmetry, absolute value knee flexion angle at IC asymmetry, and loading rate asymmetry; ***Bold**** *indicates significant interactions*

	Pre-Fatigue Mean ± SD		Post-Fatigue Mean ± SD	
	Male	Female	Male	Female
Peak Knee Flexion Angle Asymmetry	-8.42±0.72	**-8.25±0.72***	-8.75±0.72	**-8.58±0.72***
Abs Value Knee Flexion Angle at IC Asymmetry	29.7±9.86	**33.3±9.86***	30.1±9.89	**48.6±9.88***
Loading Rate Asymmetry	-5.22±6.64	4.47±6.55	-3.88±6.59	5.81±6.56

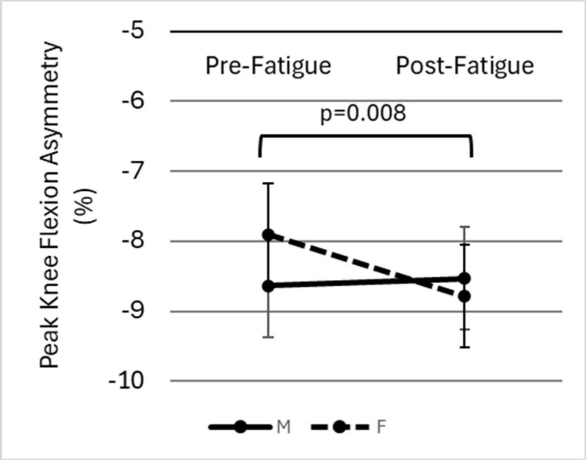


Figure 3 - Interaction plot of maximum knee angle asymmetry for male and female between fatigue conditions indicating significant differences between condition for females

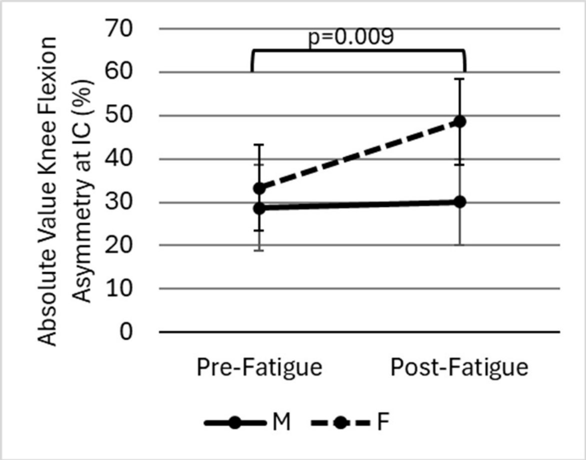


Figure 4 - Interaction plot of the absolute value knee angle asymmetry at IC for male and female between fatigue conditions indicating significant differences between condition for females

Discussion

The goal of this study was to identify differences in kinematic asymmetries between sex and fatigue in the SJ with a suspended target. It is known that kinematic measures such as maximum knee flexion angle, knee flexion angle at IC, and loading rate can be influenced by the onset of fatigue, potentially increasing the risk of injury. It was hypothesized that a significant interaction would be present between sex and fatigue with females experiencing greater asymmetry values for loading rate, maximum knee flexion angle, and knee flexion angle at IC during the post-fatigue SJ. Our hypotheses were partially supported, but not entirely.

Our findings for knee flexion angle asymmetry at IC fully supported our hypothesis, as females experienced a greater absolute value knee flexion asymmetry than males before (M=29.7±9.86, F=33.3±9.86) and after (M=30.1±9.86, F=48.6±9.88). fatigue. The amount of asymmetry increased in the fatigue condition for females, indicating a significant interaction (p<001) between females and fatigue for knee flexion angle at IC (Figure 4). Our hypothesis for maximum knee flexion angle was partially supported, as there was a significant interaction found between females and fatigue condition, with asymmetry becoming larger after fatigue (Figure 3). Our findings suggest that motor control strategies were altered due to decreased muscle strength and neuromuscular control during fatigue. As the muscles responsible for decelerating the athlete during a landing task are fatigued, there is less control over these muscles to slow the body down, increasing the knee flexion asymmetry during ground contact and the active landing. While few studies have assessed asymmetries in knee flexion angle between limbs in healthy populations during athletic tasks, these analysis have been used to understand return to sport readiness in athletes after sustaining ACL reconstruction, where smaller asymmetry values indicate more readiness to return to sport.[1] Erdman et al., for example, found larger amount of knee flexion asymmetry in athletes performing a drop vertical jump (DVJ) compared to DVJs with larger horizontal distances away from the spot of landing.[114] McPherson et al. assessed limb differences in knee flexion angles, indicating significant differences between limbs in a DVJ, however these studies used raw angle values to compare between limbs, not using a symmetry index to assess the differences.[114,42] These studies also did not include comparisons across-sex or fatigue, as the current study did.[42] In general, our finding of increased knee flexion angle asymmetries in females after fatigue suggests that females are more susceptible to injury during competitive sport conditions than males.

Our hypothesis on loading rate asymmetry was partially supported. While no significant results were found, females experienced greater asymmetry post fatigue compared to males (M=-3.88±6.55, F=5.81±6.55). The female asymmetry was positive, indicating that the dominant limb was being favored.

Overall, many statistical differences were found by assessing landing mechanics during a stop-jump before and after induced neuromuscular fatigue. Results suggest that fatigue has a significant impact on asymmetry of maximum knee flexion angle and knee flexion angle at IC during the SJ. These findings further our knowledge of differences between sexes that occur during athletic tasks that may lead to an increase in lower extremity injury, especially in the female population. In that light, the findings of this study can be used to further compare sexes in athletic-based tasks.

Chapter 4: Conclusions

Previous research has found sex-based differences in lower extremity kinetics and kinematics that are thought to heighten with the onset of fatigue. Landings that experience greater asymmetry or mechanics that result in the lower limbs being subject to an increased load are associated with an increase in injury risk.[84,81,87] During sport, females are thought to be more susceptible to lower extremity injuries due to decreased motor control[73,38,50] and increased joint laxity[38,50], which cause a more flexed landing position that is associated with knee injuries.[32] This lack of motor control is thought to heighten due to fatigue by inhibiting muscle recruitment that is necessary to create a safe landing strategy, further increasing the risk of injury.[38]

The primary objective of this study was to assess the metrics of limb stiffness and limb stiffness asymmetry to further understand how these measures could impact lower extremity injury risk. Limb stiffness was used to quantify the body's ability to resist deformation from an external force during a SJ task by calculating the ratio of the maximum resultant GRF and change in limb length. Three-dimensional motion capture was used to collect kinematics of the lower limbs, while force plates collected kinetic data. Limb stiffness and limb stiffness asymmetry using the Bilateral Asymmetry Index (BAI-1) were assessed during seven successful trials of a stop-jump (SJ) with an overhead target before and after induced fatigue. Our results did not support our original hypothesis but showed main effects of sex between post-fatigue nondominant (ND) limb stiffness, with males experiencing larger limb stiffness values than females. There were no significant differences found for limb stiffness asymmetry values.

The second hypothesis of the first aim was to assess resultant ground reaction force (rGRF) and rGRF asymmetry differences between sex and condition, as rGRF is a component of limb stiffness. Fatigue condition differences were found for both limbs, with the rGRF decreasing with the onset of fatigue. Thus, our results indicate that sex and fatigue-based differences are present in limb stiffness components.

The second objective of this study was to determine differences in knee landing mechanics asymmetry between sex after induced fatigue. Significant interactions were found between females and condition for maximum knee flexion angle asymmetry and knee flexion angle asymmetry at IC, with values becoming greater with fatigue. These results indicate that females adopt a more asymmetrical landing strategy after fatigue, further increasing the risk of injury in this population.

Comparing the results of different landing mechanics metrics, complementary results emerge. rGRF decreased with the onset of fatigue, consistent with the results that showed that the maximum knee angle increased after fatigue. Furthermore, the rGRF was found to be larger in females, aligning with the findings that females had a smaller maximum knee flexion angle than males during landings. These rGRF results are likely due to the fact that when the knee angle in the active phase of the landing is larger, there is thought to be an increase in attenuation of the force that is being placed on the lower body, spreading the force out over a longer period of time.[11]

A limitation to be acknowledged within this study is that fatigue was assessed using solely Borg's Rate of Perceived Exertion (RPE) scale, with the participant's fatigue level being based on their own discretion. While the RPE of the participants had to be within a certain level of fatigue to maintain some consistency, the participant's perception of fatigue highly influences the level of fatigue that they stated they experienced after the fatigue protocol. Potential differences in this perception and the addition of mental fatigue could ultimately have created inconsistent fatigue levels between participants. The future use of a metric such as heart rate could bridge this limitation gap, as there would have a consistent diagnostic value that would be a direct correlation to how hard the body is working during the exercises. Other limitations include the lack of analysis of frontal plane data, as the knee joint angle was assessed only in the sagittal plane. Future studies could include the use of electromyography data to provide further information at the muscular level to see how activation patterns occur before and during the landing, as the stretch-shortening cycle contains the use of preactivation of muscles before the landing and activation during the landing to produce the force needed for effective athletic tasks.

Overall, the aims of this study were to explore sex-based differences in landing mechanics and landing mechanics asymmetry during a game-like task. As limb stiffness and limb stiffness asymmetry are not values that can currently quantify injury prediction by themselves, further lower extremity mechanics data was assesed to gain a better understanding of the landing patterns. Limb stiffness and lower extremity mechanics data analyzed in this study could be used as metrics to be assessed in baseline assessments for athletic performance testing or in prospective studies as a tool to understand the current function of athletes.

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Contents