APPRAISING ANTERIOR CRUCIATE LIGAMENT INJURY RISK: SINGLE-LEGGED

JUMP-LANDING DYNAMIC POSTURAL STABILITY

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ABSTRACT

Appraising anterior cruciate ligament (ACL) injury risk is of great interest to the orthopedic and sports medicine professional. Due to the high prevalence of non-contact ACL injuries, professionals have sought to identify intrinsic and modifiable neuromuscular and biomechanical risk factors to aid in the design of injury risk screens that have the discriminatory capacity to stratify ACL injury risk and the efficiency to be implemented at-scale. Prospective studies have revealed that poor dynamic postural stability (DPS), quantified as time to stabilization (TTS), following a backwards single-legged jump-landing (BSLJL) and poor hip external rotation and abduction strength relative to body weight, quantified using a handheld dynamometer, are two risk factors for ACL injury. The purposes of this study were to assess the effects of repeated BSLJL on within-session motor learning as quantified by DPS, evaluate the reliability of DPS, and determine the relation between DPS, hip strength, and fatigue. Twenty-seven recreationally active college-aged adults (24.0 \pm 2.8 y, 1.73 \pm 0.08 m, 75. \pm 14.0 kg) were enrolled in this semirandomized, cross-over study. During visit one, subjects completed 10 BSLJL trials per leg without familiarization and had their hip external rotation and abduction strength measured. During visits two through four, subjects complete two fatigue sessions and one control session in a randomized order. During these sessions, subjects completed two sets of three BSLJL trials per leg separate by either a seated rest in a chair (control) or a short-term functional fatigue protocol (fatigue). DPS was quantified as TTS and the dynamic postural stability index (DPSI). To appropriately familiarize a subject to the BSLJL, results suggest that a minimum of six familiarization trials per leg are required to sufficiently reduce the motor learning effect. Results also indicate that TTS reliability is poor after 10 trials per leg whereas DPSI reliability is good after just two trials per leg and excellent after just six trials per leg. Finally, results indicate that following completion of a fatigue protocol, there are decrements in TTS, but not in DPSI. Further, greater hip abduction strength is associated with better DPSI when rested and fatigued, but not TTS.

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DEDICATION

This dissertation is dedicated to my wife, Anja, and my son, Carter.

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LIST OF ABBREVIATIONS

ACL	Anterior Cruciate Ligament
ACLR	Anterior Cruciate Ligament Reconstruction
RTS	Return to Sport
DPS	Dynamic Postural Stability
GRF	Ground Reaction Force
TTS	Time to Stabilization
BSLJL	Backward Single-Legged Jump-Landing
DPSI	Dynamic Postural Stability Index
MDC	Minimal Detectable Change
GRF _{vert}	Vertical Ground Reaction Force
GRF _{ML}	Medial-Lateral Ground Reaction Force
GRF _{AP}	Anterior-Posterior Ground Reaction Force
GRF _{Vert3}	Vertical Ground Reaction Force Cropped to 3 Seconds Post-Initial Contact
GRF _{ML3}	Medial-Lateral Ground Reaction Force Cropped to 3 Seconds Post-Initial Contact
GRF _{AP3}	Anterior-Posterior Ground Reaction Force Cropped to 3 Seconds Post-Initial Contact
GRF _{Vert5}	Vertical Ground Reaction Force Cropped to 5 Seconds Post-Initial Contact
GRF _{ML5}	Medial-Lateral Ground Reaction Force Cropped to 5 Seconds Post-Initial Contact
GRF _{AP5}	Anterior-Posterior Ground Reaction Force Cropped to 5 Seconds Post-Initial Contact
VSI	Vertical Stability Index
MLSI	Medial-Lateral Stability Index
APSI	Anterior-Posterior Stability Index

TTS _{Scale}	.Scaled Time to Stabilization
DPSI _{Scale}	.Scaled Dynamic Postural Stability Index
TTS _{Mean}	Rolling Scaled Time to Stabilization Mean
DPSI _{Mean}	.Rolling Scaled Dynamic Postural Stability Index Mean
TTS _{sd}	Rolling Scaled Time to Stabilization Standard Deviation
DPSI _{SD}	Rolling Scaled Dynamic Postural Stability Index Standard Deviation
TTS _{Score}	Rolling Scaled Time to Stabilization Score.
DPSI _{Score}	Rolling Scaled Dynamic Postural Stability Index.
CONTROL	.Control Session
FATIGUE	.Fatigue Session 1 or 2
REV	.Relative Error Variance
AEV	.Absolute Error Variance
MDC _{Rel}	.Relative Minimal Detectable Change
MDC _{Ab}	Absolute Minimal Detectable Change.
ICC	.Interclass Correlation Coefficient

LIST OF SYMBOLS

o	degree(s)
Δ	change
%	percent

1. INTRODUCTION

1.1. Background

Optimizing an adolescent or young adult athlete's health and wellness, performance, and quality of life are the overriding goals of the orthopedic and sports medicine professional. Musculoskeletal injury represents a substantial threat to this establishment, especially when the long-term sequela is considered. The knee is the most frequent site of athletic injury, accounting for nearly 60% of all sport-related surgeries in high-school aged athletes (Ingram et al., 2008). The anterior cruciate ligament (ACL) is a critical stabilizer of the knee. The primary function of the ACL is to control anterior translation of the tibia and the secondary function is to provide restraint to tibial rotation and varus and valgus stress (Markolf et al., 1976).

The ACL is the most frequently injured knee ligament with an estimated 200,000 to 550,000 ACL injuries occurring annually in the United States (Kaeding et al., 2017; Mall et al., 2014; Musahl & Karlsson, 2019). The incidence of ACL injury is highest for adolescents and young adults between 15 and 34 years of age (Renstrom et al., 2008) and peaks at 16 to 18 years of age, with a high proportion of these injuries occurring during athletic or physical activity participation (Musahl & Karlsson, 2019). Montalvo et al. (2019) revealed that the pooled ACL injury incidence for all athletes was 2.8% over 1 to 25 years of athletic participation and Bram et al. (2021) identified an incidence rate of 0.69 ACL injuries per 10,000 athletic exposures.

It is estimated that 40% to 70% of ACL injuries are attributed to a non-contact mechanism of injury, meaning the only appreciable external force acting on the athlete immediately prior to and at the time of injury was gravity (Boden et al., 2000; Kaeding et al., 2017; Mountcastle et al., 2007; Musahl & Karlsson, 2019). Frequent non-contact inciting events that result in ACL injury include running or jumping and suddenly decelerating or changing direction, specifically during

the eccentric or weight absorption phase of the movement within the first 0.2 seconds following initial contact of the stance leg foot with the ground (Krosshaug et al., 2007; Olsen et al., 2004; Walden et al., 2015). It is frequently purported that these ACL injuries can be prevented due to their non-contact nature, drawing substantial clinical and research interest.

The ultimate in vivo mechanism of ACL injury is a tensile stress applied to the ACL resulting in excessive strain and ultimate failure. Studies have revealed that knee abduction as well as combined knee abduction and shank internal rotation produces the greatest strain on the ACL (Bates et al., 2017). Dynamic knee valgus or pivot shift, which is the aggregation of hip internal rotation and adduction, knee rotation and abduction, and ankle eversion, is often used to describe the movement responsible of an ACL injury displayed during non-contact inciting events such as cutting, pivoting, and decelerating (Hewett et al., 2005; Hewett et al., 2016). Oftentimes, other knee joint structures in addition to the ACL are also injured, including the menisci, joint capsule, articular cartilage, subchondral bone, and other ligaments (Spindler & Wright, 2008). In fact, an isolated ACL injury occurs in less than 10% of ACL injuries (Spindler & Wright, 2008). The subchondral bone is injured in approximately 80% of ACL injuries (Speer et al., 1992), the menisci are injured in approximately 60% to 75% (Noyes et al., 1980; Piasecki et al., 2003), the articular cartilage is injured in approximately 20% to 50% (Brophy et al., 2010; Noyes et al., 1980; Piasecki et al., 2003), and a collateral ligament is injured in approximately 5 to 25% (Benjaminse et al., 2006; Spindler & Wright, 2008).

The course of treatment following ACL injury can be operative or non-operative and is highly dependent on the severity of the ACL injury, including concomitant injuries, and patient characteristics such as their pre-injury activity level and desire to return to that activity level (Musahl & Karlsson, 2019). Patients who are older, less physically active, or have no to minimal knee joint instability may choose to manage their ACL injury non-operatively with some success (Kostogiannis et al., 2007). Most adolescent and young adult athletic patients opt for ACL reconstruction (ACLR) to restore stability and integrity to the knee joint, enabling them to return to high-demand sports or activities that involve cutting, pivoting, and deceleration (Daniel et al., 1994; Kim et al., 2011). ACLR may also prevent subsequent joint damage, as an unstable, ACLdeficient knee may cause further damage to the menisci and/or articular cartilage; although, the relation between ACL-deficiency and knee osteoarthritis development is unclear (Lien-Iversen et al., 2020). Some adolescent and young adult patients may initially resist ACLR; however, these patients have a far better chance of resuming pre-injury sports participation following ACLR (Giove et al., 1983). In fact, 90% of patients less than 25 years old who initially elect non-operative management of an ACL injury eventually undergo ACLR (van der List et al., 2020). Modern ACLR for an adolescent or young adult patient is performed arthroscopically by removing the injured native ACL tissue and replacing it with a tendon autograft (Musahl & Karlsson, 2019; Spindler & Wright, 2008). Common tendon autografts include the patellar tendon, quadriceps tendon, or distal gracilis and/or semitendinosous hamstring tendons (Musahl & Karlsson, 2019; Spindler & Wright, 2008). ACL repairs, which aim to restore the continuity of the injured native ACL instead of replacing it with a tendon graft, are not commonly performed, but are of great clinical and research interest (Murray et al., 2020).

Physical rehabilitation is initiated quickly following ACLR, with the immediate goals of resolving the patient's pain, restoring normal knee joint motion, decreasing knee joint effusion, and increasing knee extensor muscle activity and strength (Cavanaugh & Powers, 2017; van Melick et al., 2016). Approximately 90% of ACLR patients restore normal joint function and can complete activities of daily living with minimal pain and limitation approximately three to four

months post-ACLR (Brewster et al., 1983; De Carlo et al., 1992; Greenberg et al., 2018; Shelbourne & Nitz, 1990). For ACLR patients who desire to return to high demand cutting, pivoting, and deceleration sports, further progression of rehabilitation is essential (Risberg et al., 2007; van Grinsven et al., 2010). These ACLR patients focus on neuromuscular training (Myer et al., 2004), which utilizes gradual exposure to higher-demand movements as well as reactional and perturbation training to improve central and peripheral nervous system capacity to control movement safely (Grooms & Myer, 2017). Ultimately, the time course of rehabilitation is highly dependent on a number of factors, including concomitant injuries and the patient's desire to return to activities with varying demands (Cavanaugh & Powers, 2017).

The optimal time frame post-ACLR in which the patient should return to sport (RTS) is highly debated with recommendations ranging from 6 months to 2 years post-ACLR (Grindem et al., 2016; Nagelli & Hewett, 2017). The majority of physical therapists indicate that ACLR patients should progress to modified sport activity, which may include agility and sport-specific drills or skills, by 4 to 5 months post-ACLR and full unrestricted RTS by 9 to 12 months post-ACLR (Greenberg et al., 2018). Around these transition times, providers administer a variety of tests to appraise the patient's status, forecast outcomes, and augment clinical decision-making (Burgi et al., 2019). The tests utilized assess multiple dimensions of rehabilitation and ACL injury risk factors including muscular strength, balance, stability, movement quality, range of motion, psychosocial, and other constructs (Burgi et al., 2019; Czuppon et al., 2014; Webster & Hewett, 2019).

Outcomes including ACL re-injury and RTS status are commonly used to evaluate the success of ACLR (Ardern et al., 2014; Wiggins et al., 2016). Re-injury to either the ipsilateral graft or contralateral native ACL and failure to RTS, in particular at the same intensity or competitive

level, after sufficient rehabilitation are considered suboptimal outcomes (Ardern et al., 2014; Wiggins et al., 2016). Despite advancements in ACL injury treatment and ACLR rehabilitation, approximately 35% of post-ACLR patients fail to RTS at the same level (Ardern et al., 2014) and 25% of young ACLR patients re-injure their ACL (Wiggins et al., 2016). One long-term negative outcome resulting from ACL injury is post-traumatic knee osteoarthritis. Subjects who sustain an ACL injury are three- to four-fold more likely to develop knee osteoarthritis than their uninjured peers (Lohmander et al., 2007) and 50% to 70% of ACLR patients develop radiographic signs of knee osteoarthritis within 12 years post-ACLR (Lohmander et al., 2007; Weninger et al., 2008). Subjects that sustain additional injuries, such as articular cartilage lesions and meniscus tears, appear to be at a greater risk for knee osteoarthritis than those that sustain an isolated ACL injury (Cox et al., 2014). Those that sustain a second ACL injury demonstrate even lower levels of knee function and a higher incidence of radiographic knee osteoarthritis after revision than those after primary ACLR (Grassi et al., 2016). Knee osteoarthritis appears to occur regardless of ACL injury treatment plan (Lohmander et al., 2007; Oiestad et al., 2010; Riordan et al., 2013; Risberg et al., 2016), which suggests that the degenerative process of knee osteoarthritis is not halted by ACLR and is catalyzed by the ACL injury itself. Knee osteoarthritis is one of the most common causes of chronic disability in adults and end stage treatment for knee osteoarthritis is a total knee arthroplasty (e.g., replacement) (Lohmander et al., 2007).

ACL injuries represent a significant fiscal burden to society. The mean lifetime cost to society for one ACL injury treated operatively or non-operatively are \$38,000 USD and \$89,000 USD, respectively (Mather III et al., 2013). Annually, ACL injury is estimated to cost between 1 and 2 billion USD (Gottlob et al., 1999; Kaeding et al., 2017), but the total lifetime cost to society for ACL injury may be much higher when the long-term sequela, including treatment of knee

osteoarthritis, is considered (Mather III et al., 2013). It has been suggested that the total direct and indirect costs of ACL injury exceed 7 billion USD annually in the United States (Musahl & Karlsson, 2019).

An emphasis has been placed on ACL injury prevention programs, which are designed to reduce the risk of sustaining an ACL injury by incorporating neuromuscular training that is similar to what would be done by ACLR patients in late stage rehabilitation into regular sport training sessions with healthy athletes (Myer et al., 2004). Key components of an ACL injury prevention program include lower-extremity and core strengthening as well as plyometric, balance, stretching, and agility training (Trojian et al., 2017). These programs have been successful, reducing the incidence of ACL injury by 50% for teams that complete it with high compliance (Huang et al., 2020; Webster & Hewett, 2018).

There are a multitude of risk factors for ACL injury (Alentorn-Geli et al., 2014; Ardern et al., 2018; Bayer et al., 2020; Bourne et al., 2019; Hewett et al., 2006). The single best predictor of a non-contact ACL injury is a history of prior ACL injury. The risk of ipsilateral graft rupture following ACLR ranges from 6 to 25% and contralateral native ACL rupture ranges from 2 to 21%, with difference in this range driven by a complex interaction between the patient's age, RTS status, and other factors (Kyritsis et al., 2016). When all ACLR patients are considered regardless of age and RTS status, approximately 15% re-injure their ACL (Wiggins et al., 2016). However, when just patients 25 years old or younger at ACLR who RTS are considered, the incidence of ACL re-injury increases to 25% (Wiggins et al., 2016). In fact, the risk for ACL re-injury for a ACLR patient is 15-fold greater in the first 12-month post-ACLR compared to healthy controls (Joreitz et al., 2016; Paterno et al., 2012, 2014). Biological sex also appears to be a strong risk factor for ACL injury as female athletes have a two- to four-fold greater risk compared to their

similarly trained male counterparts (Agel et al., 2016; Huston et al., 2000). The incidence of ACL injury is approximately 3.5% and 2.0% for female and male athletes, respectively, over a lifetime of exposures (Montalvo et al., 2019). This disparity in risk between female and male athletes is likely driven by a multitude modifiable and non-modifiable intrinsic and extrinsic risk factors (Huston et al., 2000).

Due to the high proportion of non-contact ACL injuries, research has focused heavily on identifying intrinsic and modifiable neuromuscular and biomechanical ACL injury factors. Females tend to demonstrate reduced relative muscle strength and more aberrant biomechanics associated with ACL injury (e.g., dynamic knee valgus) compared to males, although the presence of the same factors in males also increases their risk. Knee flexor and hip extensor weakness and/or under-recruitment combined with over-recruitment of the knee extensors (Myer et al., 2009; Wild et al., 2013), hip abduction and external rotation weakness and/or under-recruitment (Khayambashi et al., 2016; Zazulak et al., 2005), poor trunk or core strength and/or proprioception (Zazulak et al., 2007a, 2007b), and greater dynamic knee valgus with minimal knee flexion during force attenuation (Fagenbaum & Darling, 2003; Ford et al., 2005; Hewett et al., 2005) appear to be important intrinsic and modifiable neuromuscular and biomechanical ACL injury risk factors.

Although the definition, scope, and etiology of fatigue as related to exercise and sport performance is quite nebulous (Enoka & Duchateau, 2008; Enoka & Stuart, 1992), it does transiently reduce a subject's neuromuscular capacity to control, attenuate, and transfer force during motor control tasks (Johnston III et al., 1998; Paillard, 2012). Despite this, research has not identified a clear association between fatigue and an actual increased risk for ACL injury occurrence (Barber-Westin & Noyes, 2017; Bourne et al., 2019; Doyle et al., 2019). This may be because the ecological validity of fatigue manifested in well controlled laboratory studies is poor

and studies tend to report a heterogeneous sample of risk factors as primary outcome variables (Barber-Westin & Noyes, 2017; Bourne et al., 2019; Doyle et al., 2019). Nevertheless, it is well documented that fatigue increases the presence of aberrant neuromuscular and biomechanical factors associated with ACL injury as discussed previously (Benjaminse et al., 2008; Borotikar et al., 2008; Chappell et al., 2005; Chavez et al., 2013; Cortes et al., 2014; Kernozek et al., 2008; McLean et al., 2007; Mclean & Samorezov, 2009; Santamaria & Webster, 2010).

Developing and testing methodologies that identify subjects who are at increased risk for ACL injury has also received significant attention; however, the validity of injury risk identification is unclear and has been met with much controversy (Bahr, 2016). Specifically, in regard to post-ACLR appraisals, there has been ample research assessing their utility in relation to key re-injury and failure to RTS outcomes with equivocal findings (Burgi et al., 2019; Czuppon et al., 2014; Webster & Hewett, 2019). Ultimately, there are only a handful of studies that have demonstrated a prospective association between a proposed neuromuscular or biomechanical risk factor and ACL injury, with many of these studies utilizing advance three-dimensional motion capture laboratory equipment generally considered to be inefficient and inaccessible to most providers (Hewett et al., 2005; Paterno et al., 2010; Zazulak et al., 2007a).

Studies in 2016 by DuPrey et al. (2016) and Khayambashi et al. (2016) demonstrated a prospective relation between a neuromuscular or biomechanical risk factor and subsequent ACL injury risk. DuPrey et al. (2016) assessed dynamic postural stability (DPS), quantified using ground reaction force (GRF) from a force plate as time to stabilization (TTS), following backward, forward, lateral, and medial single-legged jump-landings in collegiate student-athletes. The authors revealed that student-athletes who subsequently sustained a non-contact ACL injury demonstrated a baseline DPS that was 50% worse following the backwards single-legged jump-

landing (BSLJL) compared to their peers who did not sustain an ACL injury (DuPrey et al., 2016). Baseline DPS following the forward, lateral, and medial jump-landing were not different between those that subsequently sustained a non-contact ACL injury and those that did not (DuPrey et al., 2016). It is unclear why only DPS following the BSLJL at baseline was associated with the odds of sustaining a subsequent ACL injury and DPS from the other directions did not, though it is likely that a BSLJL is a more demanding neuromuscular, biomechanical, cognitive, and proprioceptive task compared to the other directions (Hron et al., 2020). It should be noted that other DPS computational methods in addition to TTS are also frequently used, including the dynamic postural stability index (DPSI) (Wikstrom et al., 2005), and that they may also be associated with ACL injury risk. Khayambashi et al. (2016) assessed isometric hip external rotation and abduction strength in competitive athletes using a hand-held manual muscle tester (e.g., dynamometer). The authors revealed that athletes who subsequently sustained a non-contact ACL injury demonstrated baseline hip external rotation and abduction strength that was 22% and 19%, respectively, less compared to their peers who did not sustain an ACL injury (Khayambashi et al., 2016).

1.2. Significance of the Study

Even though the assessment of DPS may be considered lab-based, the increasing accessibility to devices that measure ground reaction force makes this assessment highly appealing. Similarly, hand-held manual muscle testers are also widely available and allow for rapid, objective assessment of strength. Together, these tests may offer an efficient and highly discriminative ACL injury risk assessment suitable for widespread adoption, yet they remain understudied.

Within a testing session subjects tend to improve their performance and consistency with repeated attempts of a motor control task (Magill & Anderson, 2010). Greater improvements are observed across attempts, especially in first few attempts, if the motor control task is novel to the subject. Familiarization trials, which are normally unmeasured, are performed at the beginning of the testing session to reduce, but likely not eliminate, this motor learning effect prior to performing the measured trials. For example, DuPrey et al. (2016) had athletes complete three familiarization trials on each leg from each jump direction prior to completing three measured trials on each leg from each jump direction. Performing an adequate number of familiarization trials to sufficiently reduce the motor learning effect is important because it will reduce an undesired source of variance (e.g., error) in task performance between subjects (Hopkins, 2000). For DPS following a BSLJL, the number of familiarization trials needed at the beginning of a testing session to sufficiently reduce the motor learning effect prior to performing measured trials is unclear.

Furthermore, subjects often perform several trials of a motor control task on multiple occasions within the same day and between days. For example, DPS could be used to monitor ACL injury risk over time or evaluate the effectiveness of an ACL injury prevention program. However, without a thorough understanding of the reliability of DPS within and between days, it is challenging to define a standard in which to gauge genuine or "real" changes in DPS that are not caused by random error (Hopkins, 2000). Determining the reliability of DPS following a BSLJL will allow for more appropriate characterizations of ACL injury risk over time.

The relation between hip external rotation and abduction strength and DPS is also unclear. It is possible that these two are correlated given poor DPS and hip strength are both associated with subsequent ACL injury (DuPrey et al., 2016; Khayambashi et al., 2016) or they are potentially uncorrelated suggesting they are indicative of independent components of ACL injury risk. Further, the relation between fatigue and DPS is also largely unexplored. It is possible that fatigue causes decrements in DPS (Benjaminse et al., 2008; Bond et al., 2020; Borotikar et al., 2008; Chappell et al., 2005; Chavez et al., 2013; Cortes et al., 2014; Kernozek et al., 2008; McLean et al., 2007; Mclean & Samorezov, 2009; Santamaria & Webster, 2010; Shaw et al., 2008; Wikstrom et al., 2004). Additionally, fatigue mimics the physiological conditions experienced during sporting participation where the majority of ACL injury occurs and elucidates deficits in neuromuscular control that may have otherwise been unobservable (Enoka & Duchateau, 2008; McLean et al., 2007; Smeets et al., 2020). A better understanding of the relation between hip strength, fatigue, and DPS would help strengthen ACL injury risk assessments.

1.3. Purposes and Hypotheses

1.3.1. Purpose and Hypothesis 1

The primary purpose of this study was to evaluate the effect of repeated BSLJL trials alternating legs on DPS, as measured by TTS and DPSI, to identify the number of familiarization trials required to sufficiently reduce the motor learning effect. The secondary purpose of this study was to assess the effect biological sex on BSLJL motor learning as measured by DPS within a single testing session. Based on previous single-legged motor control task investigations including those assessing DPS following a single-legged jump-landing (Dallinga et al., 2016; Ebben et al., 2010; Gribble et al., 2007; Hertel et al., 2000; Keklicek et al., 2019; Lephart et al., 2002; Magill & Anderson, 2010; Munro & Herrington, 2010; VanMeter, 2007; Wikstrom et al., 2006), it was hypothesized that three trials per leg would be needed to reduce the within-session learning effect and there would be no difference in motor learning between males and females.

1.3.2. Purpose and Hypothesis 2

The primary purpose of this study was to evaluate the reliability of DPS, as measured by TTS and DPSI, following a BSLJL and assess the effect of increasing the number of BSLJL trials on reliability and the minimal detectable change (MDC). Based on previous DPS investigations (Byrne et al., 2021; Colby et al., 1999; Ebben et al., 2010; Flanagan et al., 2008; Fransz et al., 2016; Fransz et al., 2015; Wikstrom et al., 2005), it was hypothesized that three trials per leg and the resulting mean value would elicit "moderate" to "good" reliability, as defined by a reliability coefficient between 0.50 and 0.90 (Koo & Li, 2016).

1.3.3. Purpose and Hypothesis 3

The primary purpose of this study was to evaluate the relation between biological sex, fatigue, hip external rotation and abduction strength, and DPS as measured by TTS and DPSI. Based on previous investigations it was hypothesized that DPS would be compromised after completion of a short, high-intensity exercise bout (Bond et al., 2020; Shaw et al., 2008; Wikstrom et al., 2004) and that subjects with greater hip strength will demonstrate superior DPS (Bandholm et al., 2011; Lephart et al., 2002; Neamatallah et al., 2020; Suzuki et al., 2015; Williams et al., 2016; Zazulak et al., 2005).

2. LITERATURE REVIEW

2.1. Anterior Cruciate Ligament Injury Risk Factors

There are multitude of risk factors for ACL injury (Alentorn-Geli et al., 2014; Bayer et al., 2020; Boden et al., 2010; Hewett et al., 2006; Huston et al., 2000; Smith et al., 2012a; Smith et al., 2012b). Risk factors can be classified as intrinsic or extrinsic. Intrinsic risk factors are internal factors particular to the subject in question, which can be further classified as modifiable and nonmodifiable risk factors. Modifiable risk factors are factors that can be changed, which may include improved muscular strength or neuromuscular control. Non-modifiable risk factors are factors that cannot be changed, such as a history of ACL injury and ACLR, age, biological sex, or ethnicity. Factors related to anatomy, joint morphology, or hormones are also considered non-modifiable. Extrinsic risk factors are external factors that apply more broadly to all subjects in a given situation. For example, some extrinsic risk factors may include the protective or sporting equipment in use, environmental factors such as weather or playing surface, or sport-specific factors such as rules and referees. There are a number of models that attempt to conceptualize the highly complex and interactive association between risk factors and the occurrence of ACL injury (Payne et al., 2016). Although these models provide a conceptual picture, they do not capture the true nature of this multifaceted phenomenon nor consider the vast array of known and potentially unknown risk factors for ACL injury. The following sections will focus on four ACL injury risk factors, which include biological sex, DPS, hip muscle strength, and fatigue.

2.1.1. Biological Sex

The female athlete is at a higher risk for ACL injury, particularly non-contact ACL injury, than their male counterparts (Agel et al., 2016; Prodromos et al., 2007). Male and female collegiate student-athletes sustain a comparable absolute number of ACL injuries; however, males account

for a larger portion of total exposures because more males participate in athletics and accumulate a larger number of exposures than females (Agel et al., 2016). When stratified by exposures, Montalvo et al. (2019) demonstrated that female athletes sustain 1.5 ACL injuries per 10,000 exposures while male athletes sustain 0.9 ACL injuries per 10,000 exposures. This equated to a relative risk 1.5-fold higher for female athletes compared to males (Montalvo et al., 2019). They further demonstrated that the disparity in ACL injury prevalence between male and female athletes was higher at the amateur level compared to intermediate and elite level of participation (Montalvo et al., 2019). Bram et al. (2021) evaluated the epidemiology of ACL injury in high-school aged athletes and demonstrated that the incidence rate for females was 0.84 ACL injuries per 10,000 exposures compared to 0.60 ACL injuries per 10,000 exposures for male athletes. The authors suggested that female high school athletes had a 1.4-fold higher risk than males at sustaining an ACL injury and that the overall risk for a multi-sport female athlete to sustain an ACL injury over an eight year high-school and collegiate career was 10% (Bram et al., 2021). Finally, Gornitzky et al. (2016) calculated the mean risk per season for an ACL injury based on an average number of exposures in a season for a given sport and revealed that a female high-school athlete has a 0.7% risk per season while male high-school athletes had a 0.4% risk per season. These meta-analyses included athletes participating in all sports, however, some studies have estimated that depending on the sport, females have approximately a three- to four-fold greater risk for sustaining an ACL injury per exposure than their male counterparts, particularly in sports such as soccer, basketball, volleyball, and gymnastics (Agel et al., 2016; Arendt et al., 1999; Prodromos et al., 2007).

This disparity in risk between female and male athletes is likely driven by a multitude factors (Huston et al., 2000). Some non-modifiable intrinsic risk factors for ACL injury that are particularly prevalent in females potentially includes larger Q angles (Hewett et al., 2006), a

narrower intercondylar notch of the distal femur (Shelbourne et al., 1998), and higher serum hormone levels of estrogen and relaxin (Park et al., 2009). Intrinsic modifiable risk factors of biomechanical or neuromuscular origin particularly prevalent in females included reduced relative muscle strength and more aberrant biomechanics during deceleration, pivoting, and landing eccentric motor control tasks, although, the presence of the same factors in males also increases their risk (Alentorn-Geli et al., 2014; Boden et al., 2010; Hewett et al., 2006). More specifically, knee flexor and hip extensor weakness and/or under-recruitment combined with over-recruitment of the knee extensors (Myer et al., 2009; Wild et al., 2013), hip abduction and external rotation weakness and/or under-recruitment (Khayambashi et al., 2007a, 2007b), and greater dynamic knee valgus with minimal knee flexion during force attenuation (Fagenbaum & Darling, 2003; Ford et al., 2005; Hewett et al., 2005) appear to be intrinsic and modifiable risk factors of neuromuscular or biomechanical origin associated with ACL injury risk that are displayed more often in females than males.

2.1.2. Dynamic Postural Stability

Joint stability is defined as the ability of the joint to remain stable when subjected to rapidly changing loads during a motor control task by producing and controlling movement through coordinated neuromuscular activity (Williams et al., 2001). Poor joint stability and neuromuscular dysfunction could result in macroscopic or whole-body postural stability deficits. Postural stability or control can be assessed in both static and dynamic conditions (Winter, 1995). This review defines static postural stability as the ability to maintain balance while the body is stationary with the feet arranged in various positions (e.g., tandem, semi-tandem, single-legged, etc.). Static postural stability may be quantified using a number of methodologies, including but not limited to

the balance error scoring system (Bell et al., 2011), Biodex balance system (Glave et al., 2016), or various center or pressure sway computations (Lehmann et al., 2017). In relation to ACL injury and ACLR, Lee et al. (2019) demonstrated that ACLR patients static postural stability measured using the Biodex balance system was not restored to pre-operative levels until one year post-ACLR and Lehmann et al. (2017) concluded that postural stability quantified using center of pressure sway during eyes open single-legged stance could differentiate between patients with ACL injury and healthy controls.

Because frequent non-contact inciting events that result in ACL injury include running or jumping and suddenly decelerating or changing direction (Krosshaug et al., 2007; Olsen et al., 2004; Walden et al., 2015), DPS may be better suited to evaluate ACL injury risk. This review defines DPS the ability to maintain balance while part of the body is in motion or when transitioning from a dynamic to a static state (Goldie et al., 1989). There are a number of motor control tasks used to assess DPS. Some motor control tasks may feature a fixed stance leg and DPS is reflective of the ability to remain balanced on the stance leg while the subject's contralateral limb is in motion and performs a dynamic task, such as during the star excursion balance test (Gribble & Hertel, 2003). Although, because non-contact ACL injury predominately occurs during the eccentric or weight absorption phase of a single- or double-legged movement (Krosshaug et al., 2007; Olsen et al., 2004; Walden et al., 2015), assessing DPS following a motor control task requiring high eccentric neuromuscular control may have higher discriminatory capacity. This review will focus exclusively on these motor control tasks, which include single- or double-legged jump-landing or single-legged cut and pivot tasks.

For single-legged jump-landing motor control tasks, versions of the multi-directional dynamic stability protocol are frequently used where subjects perform a single-legged jump-

landing from backward, forward, medial, lateral (e.g., lateral and medial refer to the direction of the jump relative to the test leg), or diagonal directions, and each direction may give unique insights into a subject's DPS capacity (Liu & Heise, 2013). Hron et al. (2020) demonstrated that DPS calculated using vertical GRF (GRF_{Vert}) was lowest (best) following forward and lateral single-legged jump-landings but medial and backward single-legged jump-landings produced slightly higher (worse) DPS. Similarly, Liu and Heise (2013) demonstrated that DPS calculated using GRF_{Vert} was lowest (best) following a forward single-legged jump-landing but medial, lateral, and backward single-legged jump-landings produced slightly higher (worse) DPS. Comparatively, Liu and Heise (2013) also demonstrated that DPS calculated using medial-lateral ground reaction force (GRF_{ML}) was higher (worse) following medial or lateral compared to forward or backward single-legged jump-landing, whereas DPS calculated using anterior-posterior ground reaction force (GRF_{AP}) was worse following forward or backward compared to medial or lateral single-legged jump-landing. Wikstrom et al. (2008) also investigated the effect of jumplanding direction on DPS and demonstrated that lateral and diagonal jump-landings produced worse DPS when computed using GRF_{ML} compared to forward jump-landings. Together, this suggests that a subject's DPS may be reflected differently depending on the horizontal GRF component used to quantify DPS and the motor control task (e.g., jump direction) used.

The difficulty of the single- or double-legged jump-landing is also frequently adjusted by requiring the subject to jump over a short hurdle placed on the edge of the force plate (DuPrey et al., 2016), touch a target hanging over the force plate at a pre-determined height based on vertical jump or standing height (McCann et al., 2018; Webster & Gribble, 2010), perform the motor control task while simultaneously performing a cognitively demanding task such as counting backwards (Dai et al., 2018), or by having the subject initiate the jump take-off but informing them

of which leg to land once they are airborne (Almonroeder et al., 2018). Some motor control tasks also feature the single- or double-legged take-off from a surface level with the force plate (DuPrey et al., 2016) or a surface that is higher than the force plate (e.g., drop jump), with the latter requiring greater neuromuscular eccentric control upon landing (Lephart et al., 2002). Finally, some motor control tasks may require the subject to jump off of both legs and land single- or double-legged whereas other tasks may require the subject to jump off of the test leg and land single-legged (DuPrey et al., 2016), again with the latter required greater neuromuscular eccentric control.

There are a number of computational methodologies used to quantify DPS (Fransz et al., 2015; Wikstrom et al., 2005). DPSI, first described by Wikstrom et al. (2005), quantifies the fluctuations (e.g., variance) of each component of GRF around a zero point, which is the subject's body weight for GRF_{Vert} and zero force for the horizontal GRF_{ML} and GRF_{AP}, with greater fluctuations representing worse DPS. The length of time post-initial contact that is used to assess these fluctuations ranges from 3 to 10 seconds, with 3 second time intervals recommended (Wikstrom et al., 2005). The fluctuations from each GRF component can be used on their own to quantify DPS, but frequently they are aggregated together to provide a single index of DPS referred to as DPSI.

Conversely, "time to stabilization" is a catch-all term used to describe a number of computational methodologies that may use different trial lengths, GRF components, input signals, stability thresholds, and stability definitions as described in depth by Fransz et al. (2015) and summarized as follows. Typical trial lengths range from 3 to 20 seconds, where the trial begins when the subject initiates the single- or double-legged jump-landing or at initial contact and concludes after the specified trial length. GRF components that can be used to calculate time to stabilization include GRF_{Vert}, GRF_{AP}, or GRF_{ML}. Input signals may include raw GRF (either

unfiltered or filtered), a sequential average of the GRF signal such that the new signal average is recalculated in a pointwise fashion after the addition of each new GRF digitized data point, a unbounded third order polynomial fit to the post-initial contact raw GRF such that it represents a smooth decay in the raw GRF post-initial contact, or a moving root mean square window to also represent a smooth decay in the raw GRF post-initial contact. Thresholds to define when stability has been achieved include when the input signal reaches or reaches and remains (see below) within the subject's body weight plus or minus 5% (only for GRF_{vert}), the mean overall input GRF for the entire trial plus or minus a pre-determined standard deviation (e.g., 0.25 standard deviations), the minimal to maximal range of the input GRF observed during quite stance (e.g., final 5-10seconds of a 20 second trial), or the mean range of the input GRF for quite stance (e.g., final 5 – 10 seconds of a 20 second trial) plus or minus a pre-determined standard deviation (most often 3.0 standard deviations). Finally, stability definitions, or the "time" part of "time to stabilization," may be defined as the time required for the input signal to reach the stability threshold or the time required for the input signal to reach and remain within the stability upper and lower bound thresholds for either the remainder of the trial or a predetermined length of time (e.g., one second). Note that from this point forward, "time to stabilization" will refer to one of the most popular computational methodologies and the one used by DuPrey et al. (2016) in which the input signal is raw (filtered) GRF_{Vert}, the stability threshold is the subjects body weight plus or minus 5%, and the stability definition is the time required for the signal to reach and remain within the threshold for the remainder of the trial. Nevertheless, DPS following a jump-landing, however quantified, represents a subject's capacity to efficiently and quickly decelerate their center of mass using eccentric neuromuscular control to achieve a motionless state.
There are several studies that utilized DPS to assess ACL injury risk, post-ACLR neuromuscular dysfunction, or ACL deficient neuromuscular dysfunction (Colby et al., 1999; DuPrey et al., 2016; Webster & Gribble, 2010). DuPrey et al. (2016) assessed DPS following single-legged jump-landings from forward, backward, medial, and lateral jump directions in 278 collegiate student-athletes at baseline and then prospectively monitored them for ACL injury for four years (DuPrey et al., 2016). Nine athletes sustained a non-contact ACL injury during the monitoring period. Analysis revealed that poor DPS following the BSLJL was associated with subsequent ACL injury. Webster and Gribble (2010) assessed DPS following a forward singlelegged jump-landing in twelve female college athletes with a history of ACLR (mean 2.5 y post-ACLR) and twelve female college athletes matched by age and sport without a history of ACLR. The athletes with a history of ACLR demonstrated worse DPS than the healthy controls, potentially suggesting that ACL injury results in long-term decrements to DPS. Finally, Colby et al. (1999) assessed DPS following a forward single-legged jump-landing in adults 4 to 6 months post-ACLR, adults with ACL deficiency, and adults without a history of ACL injury. The authors found that DPS had the potential to distinguish between the injured and uninjured leg for the ACLR and ACL deficient subjects and suggested that DPS could be used to monitor progress during rehabilitation.

Several studies have compared DPS between healthy males and females. Ebben et al. (2010) evaluated DPS in college aged male and female athletes following a single-legged jumplanding. Although the authors did not directly compare men and women, mean DPS values tended to suggest fairly equivalent DPS between biological sexes. Lephart et al. (2002) evaluated kinematics and DPS in female collegiate athletes and matched males during a single-legged drop jump-landing and forward single-legged jump-landing and found no evidence of a difference in DPS between males and females on either task. In contrast to these studies, Wikstrom et al. (2006) evaluated DPS between males and females following a forward single-legged jump-landing and demonstrated that females had worse DPS compared to males, whereas Dallinga et al. (2016) evaluated DPS between males and females following single-legged jump-landings and revealed that males had worse DPS than females. The effect of biological sex on DPS is unclear, but it should be noted that the studies utilized various DPS computational methodologies and motor control tasks making it difficult to compare findings across studies.

2.1.3. Proximal Hip Strength

Proximal hip weakness, and in particular hip external rotation weakness and underrecruitment, has been identified as a risk factor for non-contact ACL injury (Khayambashi et al., 2016; Lephart et al., 2002; Zazulak et al., 2005). This may be because adequate hip strength is required to resist hip internal rotation and adduction, which are two components of the dynamic knee valgus mechanism, during motor control tasks that require eccentric control (Powers, 2010). Khayambashi et al. (2016) assessed the isometric hip external rotation and abduction strength of 501 male and female athletes using a hand-held dynamometer and then prospectively monitored the athletes for one year for ACL injury. Fifteen non-contact ACL injuries occurred during the monitoring period. Analyses revealed that hip strength was associated with ACL injury. For every 1% decrease in force relative to body mass the odds of ACL injury increased 23% and 12% for external rotation and abduction strength, respectively. The authors then established optimized clinical cutoffs for the strength measures to predict ACL injury. They found that an external rotation strength less than 20.3% of body weight has a sensitivity of 93% and a specificity of 59% to predict ACL injury and an abduction strength less than 35.4% of body weight had a sensitivity of 87% and a specificity of 65% to predict ACL injury.

Steffen et al. (2016) evaluated female handball and soccer player's baseline isokinetic knee extension and flexion strength, knee extensor-to-flexor strength ratio, isometric hip abduction strength, and one repetition maximum leg press strength and then prospectively monitored the athletes for occurrence of a new ACL injury. Contrary to the study by Khayambashi et al. (2016), the authors of this study did not find an association between any of the five strength tests, including hip abduction strength, and the occurrence of a new non-contact ACL injury. However, in this study, the mean time from baseline testing to ACL injury was 1.8 years, which is much longer than the 1 year between baseline testing and cessation of ACL injury surveillance used by Khayambashi et al. (2016).

Other studies have assessed the relation between lower-extremity strength and DPS. Although these studies did not evaluate proximal hip strength, the strength of one muscle group tends to correlate with the strength of other muscle groups within a subject, potentially suggesting that similar relations between DPS and hip strength would also be observed. Lephart et al. (2002) evaluated knee extensors and flexor strength, kinematics, and DPS in female collegiate athletes and matched males during a single-legged drop jump-landing and forward single-legged hop. The authors demonstrated that females had lower strength normalized to body mass for the knee extensors and flexors, but there was no difference in DPS between sexes. The authors did find several differences in kinematics between genders. Females displayed less knee flexion at initial contact and less knee flexion angular excursion during the eccentric phase of the jump-landings. Therefore, sufficient strength is needed to decelerate the center of mass during jump landing through eccentric control of the knee extensors and potentially the biarticulate knee flexors and hip extensors, and females potentially circumvent this strength threshold by stiffening at the knee during jump-landing. Although this compromise did not result in worse DPS in this study, it is possible that other jump-landing tasks or the presence of additional risk factors may result in compromised DPS. Contrary to this study, Williams et al. (2016) assessed DPS following a forward single-legged jump-landings in 94 male military members and evaluated their knee flexion and extension strength. The authors demonstrated that soldiers with greater knee extension and flexion strength, ankle range of motion, and ankle inversion and eversion strength demonstrated superior DPS.

There have also been several studies to investigate the association between proximal hip strength or neuromuscular activation and lower-extremity biomechanics that may be related to ACL injury risk besides poor DPS, such as greater knee stiffness or frontal plane knee motion and moments. Zazulak et al. (2005) investigated proximal hip and thigh neuromuscular activity immediately before and after a double-legged drop jump-landing in collegiate male and female athletes. The authors demonstrated that females tended to have greater rectus femoris activity immediately prior to landing but lower gluteus maximus activity immediately post-landing compared to males. Therefore, females appeared to adopt a strategy in which they under-recruit the proximal hip musculature and over-recruit the knee extensors to stiffen their knee joint immediately prior to landing, which potentially results in the decreased knee flexion angle at initial contact and knee flexion angular excursion after landing as demonstrated by Lephart et al. (2002). Suzuki et al. (2015) assessed the effect of proximal hip strength on knee kinematics in male and female collegiate athletes following a single-legged medial drop jump-landing. The authors revealed that females with greater hip abduction, extension, and external rotation strength displayed lower knee abduction (e.g., dynamic knee valgus) angles during jump-landing, although the same correlation was not elucidated in males. Similarly, Bandholm et al. (2011) evaluated the effect of hip abduction and external rotation strength in recreationally active females on frontal

plane knee motion during drop jumps. The authors of this study revealed that females with lower hip external rotation strength displayed greater frontal plane knee motion (e.g., dynamic knee valgus) during landing, but hip abduction strength was unrelated to frontal plane knee motion. Finally, Neamatallah et al. (2020) examined the relation between proximal hip muscle activity and strength and lower-extremity biomechanics during single-legged jump-landings in recreationally active males and females. The authors demonstrated that females' gluteus medius and gluteus maximums activity and hip abduction strength was strongly related to knee abduction moments and angles during the jump-landing, whereas the relations between proximal hip muscle strength and activity and knee abduction moments and angles were not as clear. Together, these studies suggest that adequate proximal hip strength is essential in controlling frontal plane knee motion during tasks that require eccentric control and that there may be biological sex specific relations between strength and kinematics.

2.1.4. Fatigue

Although the etiology of fatigue as related to exercise and sport performance is unclear (Enoka & Duchateau, 2008; Enoka & Stuart, 1992), fatigue does transiently reduce a subject's neuromuscular capacity to control, attenuate, and transfer force during motor control tasks (Johnston III et al., 1998; Paillard, 2012). Despite this, research has not identified a clear association between fatigue and an actual increased risk for ACL injury occurrence (Barber-Westin & Noyes, 2017; Bourne et al., 2019; Doyle et al., 2019). This may be because the ecological validity of fatigue manifested in well controlled laboratory studies is poor and studies tend to report a heterogeneous sample of risk factors as primary outcome variables (Barber-Westin & Noyes, 2017; Bourne et al., 2019). Nevertheless, it is well documented that fatigue

increases the presence of aberrant neuromuscular and biomechanical factors associated with ACL injury.

Wikstrom et al. (2004) assessed DPS following a forward single-legged jump-landing in twenty healthy college aged males and females before and after an isokinetic and fatigue protocol. The isokinetic fatigue protocol consisted of repeated plantar- and dorsi-flexion concentric contractions until the maximal torque during a repetition decreased below 50% of their peak torque. The fatigue protocol consisted of a series of six agility drills performed in succession until the time to complete all six drills increased by 50% above the time to complete the first round. The authors revealed that both fatigue protocols caused a similar decrement in DPS. Bond et al. (2020) assessed DPS after a forward single-legged jump-landing following repeated bouts of aerobic exercise in temperate and hot environmental conditions in six recreationally active high-school males. The males completed two, 60-minute bouts of sub-maximal aerobic exercise with 60 minutes of rest between bouts in both hot (35°C) and temperate (22.2°C) conditions. The authors revealed that fatigue tended to cause a decrement in DPS and that repeated bouts of exercise in the hot environment tended to cause greater decrements in DPS than exercise bouts in the temperate environment. Shaw et al. (2008) evaluated the effect of fatigue on DPS after a forward singlelegged jump-landing in female collegiate volleyball players. Subjects completed a functional fatigue protocol that consisted of agility drills, stationary lunges, and counter movement vertical jumps. The authors found that subjects DPS increased (worsened) from pre- to post-fatigue.

There have also been several studies to investigate the association between fatigue and lower-extremity biomechanics that may be related to ACL injury risk besides poor DPS. Cortes et al. (2012) evaluated the effect of fatigue on lower-extremity biomechanics during a side-step cut in female collegiate athletes and demonstrated that fatigue caused aberrant biomechanics including

reduced knee flexion angles (straighter leg), a larger knee abduction moment (e.g. dynamic knee valgus moment), and a smaller hip abduction angle (closer to neutral). Benjaminse et al. (2008) examined the effect of fatigue on lower-extremity biomechanics during a single-legged jump-landing in recreationally active males and females and found that the subjects exhibit less knee flexion at initial contact (straighter leg), but there was no difference in the change in kinematics from before to after fatigue between biological sexes. Chappell et al. (2005) evaluated the effect of fatigue on lower-extremity biomechanics during a single-legged jump-landing in reactional male and female athletes and demonstrated that both male and female subjects displayed greater peak proximal tibia anterior shear forces, increased knee abduction moments, and decreased knee flexion angles when fatigue, but the change in these parameters from before to after fatigue was similar for male and female subjects.

3. METHODS

3.1. Research Design

This study used a crossover, semi-randomized design. Subjects completed four visits to the laboratory (Figure 1). Visit one, referred to as the learning effect session, always consisted of the same procedures for all subjects. During this learning effect session, subjects completed informed consent and baseline forms, hip abduction and external rotation strength assessments, ten trials per leg of the BSLJL, and a vertical jump test. The next three visits consisted of one control session and two fatigue sessions. During these visits, subjects completed two sets of three trials per leg of the BSLJL separated by either a seated rest period or a fatigue protocol. The order of the control and fatigue sessions over visits two, three, and four was randomized to control for an order effect.

Figure 1

Schematic illustrating the study visits and session types

Learning Effects Session Informed Consent, Contact Information, Physical Activity Readmens: Questionnaire, and Heath Nistory Assessment Form - Measurements - Body Mass - Bidyt Leg Length - Right Shank Length - Hip External Rotation - Hip Adduction - Hip Adduction - Nip Adduction - Di Backward Sangle-Length Jump-Landing Trials Per Leg - Vertical Reach and Jump Assessment Content Aessage - Guided 10 Minute Warm-Up - 3 Backwards Single-Legged Jump Landing Trails Per Leg - Rest Seated In Chair For S Minutes - 3 Backwards Single-Legged Jump Landing Trails Per Leg

Fatigue Session - Guided 10 Minute Warm-Up - 3 Bachwards Single-Legged Jun Landing Trials Per Leg - Short-Term Fatigue Protocol - 3 Backwards Single-Legged Jun Landing Trials Per Leg

Fatigue Session - Guided 10 Minute Warm-Up - 3 Backwards Single-Logged Jum Landing Trials Per Leg Short-Term Fatigue Protocol - 3 Backwards Single-Logged Jum Landing Trials Per Leg Fattgue Session - Guided 10 Minute Warm-Up - 3 Backwards Single-Legged Jump-Landing Trisls Per Leg - Short-Term Fattgue Protocol - 3 Backwards Single-Legged Jump-Landing Trisls Per Leg

Fatigue Session - Guided 10 Minute Warm-Up - 3 Backwards Single-Legged Jump-Landing Trials Per Leg - Short-Term Fatigue Protocol - 3 Backwards Single-Legged Jump-Landing Trials

Control Session Guided 10 Minute Warm-Up 3 Backwards Single-Legged Jumpanding Trials Per Leg Reit Seated in Chair For 5 Minutes 3 Backwards Single-Legged Jumpanding Trials Per Leg Eatigue Session Guided 10 Minute Warm-Up 3 Backwards Single-Legged Jumpanding Trials Per Leg Short-Term Fatigue Protocol 3 Backwards Single-Legged Jumpanding Trials Per Leg

Control Session Guided 10 Minute Warm-Up 3 Backwards Single-Legged Jump anding Trials Per Leg Rest Seated In Chair For 5 Alnutes 3 Backwards Single-Legged Jump

Canada a succession of the

Guided 10 Minute Warm-Up
 Backwards Single-Legged Jump
 Landing Trials Per Leg
 Short-Term Fatigue Protocol
 Backwards Single-Legged Jump
Landing Trials Per Leg

3.2. Subjects

Recreationally active males and females between 12 and 30 years of age were included in this study as this demographic represents those who sustain the highest proportion of ACL injuries (Renstrom et al., 2008). Subjects with prior ligamentous, bony, or other soft tissue lower-extremity procedures, an orthopedic issue exacerbated by exercise, acute fracture, tumor, or infection, unfavorable cardiovascular responses to exercise, a neurological condition that effects the activation of skeletal muscle or balance, and diabetes were excluded from the study. Additionally, active smokers or those that have smoked in the past 6 months, pregnant females, and cognitively impaired adults were also excluded from this study.

3.3. Protocol

3.3.1. Visit One - Learning Effect Session

Subjects were informed about the research project and be given the opportunity to ask a member of the research team questions prior to providing their informed, written, voluntary consent. Each subject completed a contact information sheet, physical activity readiness questionnaire, and a health history assessment form, which were used to verify they met all eligibility criteria and collect important baseline demographic information. Subjects then had their anthropometrics recorded, which included height (m), body mass (kg), right leg length (m) measured from the anterior superior iliac spine to medial malleoli measured with a tape measure while the subject is in the supine position, right shank length (m) measured from the lateral knee joint line to lateral malleoli measured with a tape measure while the subject is in the supine position (Myer et al., 2010), and leg dominance (left or right) as the leg the subject indicated they would kick a soccer ball with. Leg and shank length were only be assessed on the right leg as these measures were not expected to be appreciably different than the left leg in this healthy population.

Subjects then completed a guided 10-minute warm-up consisting of light aerobic exercise, dynamic stretching, and plyometrics (Table 1).

Table 1

Warm up procedure

Exercise	Notes
Jogging	Approximately 200 yards or 2 minutes
Lunges	4 per leg
Standing Knee Extensor Stretch	4 per leg, hold for 2 seconds
Standing Hip External Rotator Stretch	4 per leg, hold for 2 seconds
Standing Hip Extensor/Knee Flexor Stretch, Straight Leg Kicks	4 per leg
Standing Hip Extensor/Knee Flexor Stretch, Knee Hugs	4 per leg, hold for 2 seconds
Jumping Jacks	10
Single Leg Vertical Hops	5 per leg, hands on hips
Single Leg Horizontal Hops Medial-Lateral	5 per leg, hands on hips
Single Leg Horizontal Hops Anterior-Posterior	5 per leg, hands on hips

The subject was then assessed for bilateral hip external rotation and abduction strength using the procedures described by Khayambashi et al. (2016). For isometric hip external rotation, the subject sat on the edge of an exam table with their knees flexed to 90° (Figure 2). The hand-held manual muscle tester was placed proximal to the medial malleolus of the test leg. The subject was then given an audible "three, two, one, go" command from the investigator. The subject then performed a maximal voluntary isometric hip external rotation contraction. The manual muscle

tester emitted an audible "beep" when the subject first exceeded 22 N of force and data was sampled for three seconds after this instance. At the conclusion of the 3 seconds, the manual muscle tester emitted another audible "beep" indicating that the trial was over. Three trials were performed per leg with 15 seconds of rest between trials.

Figure 2

Isometric hip external rotation strength test



For isometric hip abduction, the subject laid on their side with their back supported by a firm surface (Figure 3). The subject abducted their hip approximately 30°. The hand-held manual muscle tester was placed just proximal to the lateral femoral epicondyle of the test leg. The subject was then given an audible "three, two, one, go" command from the investigator. The subject then performed a maximal voluntary isometric hip abduction contraction. Again, the manual muscle tester emitted an audible "beep" when the subject first exceeded 22 N of force and data was sampled for three seconds after this instance. At the conclusion of this 3 seconds, the manual muscle tester emitted another audible "beep" indicating that the trial is over. Three trials were performed per leg with 15 seconds of rest between trials.

Figure 3

Isometric hip abduction strength test



The BSLJL used in this study was identical to the protocol used and described by DuPrey et al. (2016), which was adopted from Liu and Heise (2013). Subjects started the jump-landing task by standing on two feet on the floor directly next to the force plate with their back facing the force plate and their hands on their hips (Figure 4). A 0.05 m tall hurdle was placed parallel with the edge of the force plate nearest to the subject's starting position. This hurdle was used to normalize the minimal foot clearance off the ground required to successfully complete the task. An investigator gave the subject an audible "three, two, one, go" command. GRF data started recording when the investigator said "go" and was recorded for 12 seconds, which was decided a priori based on the assumption that most subjects would initiate the jump and make initial contact within the first 2 seconds leaving ten additional seconds of GRF data post-initial contact. When the subject heard "go," they lifted the non-test leg off the ground and jumped off the test leg backwards over the hurdle and onto the force plate. The subject was instructed to land on the force plate on the test leg with their eyes focused forward and their hands on their hips, stabilize as quickly as possible, and remain motionless until the investigator indicated the trial was over. The trial was over once the 12 seconds elapsed. Trials were performed barefoot to minimize the

stability provide by a shoe. Following the initial contact, the subject was permitted to hop or shuffle on their test leg to obtain stability if their test leg foot did not contact any surface except the force plate. Trials where the subject removed their hands from their hips upon landing, touched their non-test leg or any other body part to the ground, or contacted their test leg to any surface besides the force plate were considered "failed trials." These failed trials were documented, but not repeated.

Figure 4

The starting position (left) and finishing position (right) for the backwards single-legged jump-landing task





The subject had the BSLJL demonstrated to them by an investigator. The subject had the opportunity to ask the investigator questions about the task. Since a purpose of this research study was to examine the within day learning effects of repeatedly performing the BSLJL on DPS, there were no familiarization or practice trials performed and all trials were measured. Prior to performing the first trial, the subject stepped onto the force plate and remained as motionless as possible and GRF data was sampled for 10 seconds to obtain the subject's body weight (BW; N). The subject then performed ten trials per leg, or twenty total trials, by alternating between the dominant and non-dominant leg to avoid an acute fatigue effect. There was 15 seconds rest

between trials, or 30 seconds of rest between trials performed on the same leg. The leg that performed the first trial was randomized to control for an order effect.

To conclude visit one, the subject had their maximal vertical reach and jump measured to standardize the jump height used for the countermovement jumps in the fatigue protocol, which is explained in detail below (Chappell et al., 2005; Cortes et al., 2012). The subject stood directly under a Vertec and extended their dominant arm overhead as high as possible keeping their heels on the floor. The subject's vertical reach was measured as the maximum height (m) they could reach with their fingertips. The subject then performed a maximal effort countermovement jump reaching upwards with their dominant hand and jump height (m) was recorded. Each subject was given three attempts with 30 seconds of rest between attempts. The subject's maximal vertical jump was calculated as the difference between their vertical reach and best jump height (best jump height – vertical reach; m). The vertical jumps were performed shod.

3.3.2. Visit Two, Three, and Four

Visits two, three, and four consisted of one control session and two fatigue sessions. The order of these sessions over visits two, three, and four were randomized to control for an order effect.

3.3.2.1. Control Session

The subject arrived at the laboratory and was outfitted with a heart rate monitor (H9, Polar USA, Bethpage, NY, USA) worn around the upper torso. No heart rate data was recorded during the control session but was recorded during the fatigue session, so the subject wore it during the control session for consistency. Prior to performing the BSLJL, the subject completed a guided 10-minute warm-up consisting of light aerobic exercise, dynamic stretching, and plyometrics that was identical to the protocol completed during visit one. The subject then performed three practice

trials each on the dominant and non-dominant leg, for a total of six practice trials of the BSLJL while barefoot to re-familiarize themselves with the task.

Prior to performing the first measured trial, the subject stepped onto the force plate and remain as remained as motionless as possible and ground reaction force data was sampled for 10 seconds to obtain the subject's body weight (BW; N). The subject then performed three measured trials each on the dominant and non-dominant leg, for a total of six trials, alternating the testing leg (PRE). The subject was given 15 seconds of rest between trials, or 30 seconds of rest between trials performed on the same leg. The leg that performed the first trial was randomized to control for an order effect. The subject then rested in the seated position in a chair for 5 minutes, which is approximately equal to the time required to complete the fatigue protocol explained in detail below. The subject then repeated the BSLJL procedure (POST). Again, the subject performed three trials each on the dominant and non-dominant leg, for a total of six trials, alternating the testing leg (PRE). The subject got 15 seconds of rest between trials, or 30 seconds of rest between trials performed on the same leg. The leg that performed the first POST trial was the same leg the performed the first PRE trial.

3.3.2.2. Fatigue Sessions

The subject arrived at the laboratory and was outfitted with a heart rate monitor (H9, Polar USA, Bethpage, NY, USA) worn around the upper torso. The heart rate data was used to determine the degree of fatigue (e.g., percent of estimated heart rate maximum) achieved by the subject during the fatigue protocol. Prior to performing the BSLJL, the subject completed a guided 10-minute warm up consisting of light aerobic exercise, dynamic stretching, and plyometrics that was identical to that used during visit one. The subject then performed three practice trials each on the

dominant and non-dominant leg, for a total of six practice trials of the BSLJL while barefoot to refamiliarize themselves with the task.

Prior to performing the first measured trial, the subject stepped onto the force plate and remain as remained as motionless as possible and GRF data was sampled for 10 seconds to obtain the subject's body weight (BW; N). The subject then performed three measured trials each on the dominant and non-dominant leg, for a total of six trials, alternating the testing leg (PRE). The subject got 15 seconds of rest between trials, or 30 seconds of rest between trials performed on the same leg. The leg that performed the first trial was randomized to control for an order effect. After completing the sixth trial, the subject had 1 minute to put their shoes on.

The subject then completed a fatigue protocol similar to the procedure described by Cortes et al. (2012). This protocol consisted of a series of four exercises, which included step-ups on a 0.3m tall box, an "L-drill," five countermovement vertical jumps reaching to 80% of the subject's maximal vertical jump height that was identified during visit one, and agility drills on an agility ladder. The fatigue protocol started by having the subject perform a series of step-up movements onto a 0.3-m box for 20 seconds (McLean et al., 2007). Immediately after, the subject performed one repetition of an L-drill. This drill is performed by placing three cones on the ground in an "L" formation 4.5 m apart. The subject then sprinted around the cones in a standardized order making an "L" shape (Sierer et al., 2008). Following the L-drill, the subject completed five countermovement vertical jumps reaching up to touch a marker set at 80% of their maximal vertical jump height identified during visit one. Finally, the subject completed agility drills on an agility ladder, which consisted of sprinting forwards, backwards, or sideways and placing each foot in a designated section of a 5-yard ladder with rungs approximately sixteen inches apart. This entire sequence of four exercises was completed four times, with no rest between rounds. Heart

rate was monitored throughout the fatigue protocol using the heart rate monitor, which streamed data via Bluetooth to an Ipad (Apple Inc., Cupertino, CA, USA) and was visualized using the Polar Beat App (Polar USA, Bethpage, NY, USA). The subject was considered fatigued if their heart rate exceeded 85% of their estimated maximal heart rate (220 – age in years; beats·minute⁻¹) at any point during the fatigue protocol. The protocol took approximately 5 to 7 minutes to complete.

At the conclusion of the fatigue protocol, the subject quickly took off their shoes. The subject then repeated the BSLJL procedure (POST), with the first trial initiated within 30 seconds of completing the fatigue protocol. Again, the subject performed three trials each on the dominant and non-dominant leg, for a total of six trials, alternating the testing leg (PRE). The subject got 15 seconds of rest between trials, or 30 seconds of rest between trials performed on the same leg. The leg that performed the first POST trial was the same leg the performed the first PRE trial.

3.4. Instrumentation

A Lafayette hand-held manual muscle tester (Model 01165, Lafayette Instrument, Lafayette, IN, USA) sampling at 40 Hz was used to assess hip external rotation and abduction strength. Peak force (N), defined as the greatest force value recorded at any point during the three second trial, was recorded and averaged over the three trials per leg per assessment (e.g., external rotation and abduction). Relative peak force was then be calculated as the ratio of peak force (N) to body weight (BW; N) (N·BW⁻¹) and reported as a percentage, identical to how Khayambashi et al. (2016) did. Relative peak torque was then calculated as the product of peak force (N) and the moment arm (m), which was estimated as shank and thigh length for hip external rotation and abduction, respectively, to body mass (kg) (N·m·kg⁻¹). Relative peak torque was calculated as it provides and additional degree of allometric scaling (Bazett-Jones et al., 2011).

An in-ground 0.6 x 0.9 m three-dimensional force plate (Bertec FP6090-15, Columbus, OH, USA) level with the surrounding floor was used to collected ground reaction force (GRF) data during the single-legged jump-landings. The analog signal from the force plate was connected to an amplifier (Bertec AM6800, Columbus, OH, USA) with a gain setting of one. Vertical ground reaction force (GRF_{Vert}), medial-lateral ground reaction force (GRF_{ML}), and anterior-posterior ground reaction force (GRF_{AP}) were sampled at 1 kHz.

3.5. Dependent Variable Calculation

Data was processed using a custom written MATLAB program (R2021a, MathWorks, Natick, MA, USA). Raw GRF_{Vert}, GRF_{AP}, and GRF_{ML} were filtered post-hoc using a second order 12 Hz low-pass Butterworth filter (Ross et al., 2005; Webster & Gribble, 2010). All further use of GRF data utilized filtered GRF_{Vert}, GRF_{AP}, and GRF_{ML}. It should be noted that various filters have been used to process GRF data for DPS and it is recognized that different order and frequency low pass filters will elicit different DPS (Fransz et al., 2015). The subject's body mass (N·9.81⁻¹; kg) was calculated as the mean GRF_{Vert} from the ten second trial performed at the beginning of each visit.

Initial contact was defined as the instant GRF_{Vert} first exceeded 20 N. GRF data was cropped into 3- and 5-second post-initial contact time frames (GRF_{Vert3} , GRF_{AP3} , and GRF_{ML3} and GRF_{Vert5} , GRF_{AP5} , and GRF_{ML5} , respectively). TTS (Δ time; s) was calculated using GRF_{Vert5} as the length of time in seconds required for GRF_{Vert5} to reach and then remain between 95% and 105% of the subject's body weight (GRF_{Vert5} ·body weight in N⁻¹; %) for the remainder of the trial (DuPrey et al., 2016). Dynamic postural stability indices for GRF_{Vert} , GRF_{ML} , and GRF_{AP} were calculated using the methodology first described by Wikstrom et al. (2005) and later modified by Wikstrom et al. (2010) and Dallinga et al. (2016) using GRF_{Vert3} , GRF_{ML3} , and GRF_{AP3} . Stability indices (standard deviation around a zero point) for GRF_{ML} (MLSI), GRF_{AP} (APSI), and GRF_{Vert} (VSI) reflect the average magnitude of the GRF_{Vert3}, GRF_{ML3}, and GRF_{AP3} vectors around zero points for MLSI and APSI and the subject's body weight for VSI (Figure 9). A composite score of MLSI, APSI, and VSI called the "dynamic postural stability index" (DPSI) was also calculated. These dynamic postural stability indices were calculated using the following equations (Dallinga et al., 2016; Wikstrom et al., 2010) where body weight is the subject's body weight in N and samples is the number of GRF data points included (e.g., 3,000 for a 3 second post-initial contact time frame if recorded at 1 kHz as done here):

$$Medial - Lateral Stability Index = \sqrt{\sum ((0 - GRF_{ML}) \cdot Body Weight^{-1})^{2} \cdot Samples^{-1}}$$

$$Anterior - Posterior Stability Index = \sqrt{\sum ((0 - GRF_{AP}) \cdot Body Weight^{-1})^{2} \cdot Samples^{-1}}$$

$$Vertical Stability Index = \sqrt{\sum ((GRF_{Vert} - Body Weight) \cdot Body Weight^{-1})^{2} \cdot Samples^{-1}}$$

 $Dynamic \ Postural \ Stability \ Index = \sqrt{((\sum (0 - Fx)^2 + \sum (0 - Fy)^2 + \sum (Body \ Weight - Fz)^2) \cdot Body \ Weight^{-1})) \cdot Samples^{-1}}$

Data for TTS and DPSI was entered into a data matrix. Because "failed" trials were not repeated, cells corresponding to failed trials were left blank. Cells corresponding to TTS were also left blank for trials in which the stability threshold, defined as reaching and remaining between 95% and 105% of the subject's body weight for the remainder of the trial, was not achieved within the 5-second post-initial contact period; although, the cell for DPSI still did have a value in this instance.

3.6. Statistical Analyses

3.6.1. Purpose and Hypothesis 1

The primary purpose of this study was to evaluate the effect of repeated BSLJL trials alternating legs on DPS, as measured by TTS and DPSI, to identify the number of familiarization trials required to sufficiently reduce the motor learning effect. The secondary purpose of this study was to assess the effect biological sex on BSLJL motor learning as measured by DPS within a single testing session. Based on previous single-legged motor control task investigations including those assessing DPS following a single-legged jump-landing (Dallinga et al., 2016; Ebben et al., 2010; Gribble et al., 2007; Hertel et al., 2000; Keklicek et al., 2019; Lephart et al., 2002; Magill & Anderson, 2010; Munro & Herrington, 2010; VanMeter, 2007; Wikstrom et al., 2006), it was hypothesized that three trials per leg would be needed to reduce the within-session learning effect and there would be no difference in motor learning between males and females.

Data collected during visit one was used to evaluate the hypotheses. The independent categorical variables of this study were leg (e.g., dominant or non-dominant), sex (e.g., male and female), and trial (e.g., 1, 2,...10). The dependent continuous variables were TTS and DPSI. Statistics were completed using R v. 4.0.5 (R Core Team, 2019) and accompanying packages "lme4" v. 1.1-28 (Bates et al., 2022) and "emmeans" v. 1.7.2 (Lenth et al., 2022). Descriptive statistics including means, 95% confidence intervals, medians, quartiles, minimum (25th Percentile – (1.5 · Interquartile Range)), and maximum (75th Percentile + (1.5 · Interquartile Range)) as well as normal Q-Q plots and histograms were used to initially explore the data.

A series of linear mixed effects models were then conducted to examine the magnitude of change in TTS and DPSI over trials by biological sex and leg. In these models, a full factorial of trial, biological sex, and leg were entered as fixed effects and each subject was assigned a random intercept. Estimated marginal means were then computed at each level of the fixed effects. If appropriate, Bonferroni corrected post-hoc comparisons were used to assess the trial-to-trial change (e.g., trial 2 - trial 1, 3-2...10-9) in the dependent variables by biological sex and leg. Additionally, the linear and quadratic contrasts for each biological sex and leg were also computed

to evaluate underlying linear and quadratic changes in the dependent variables over trials. Significance was set at p < .05.

TTS and DPSI were then separately rescaled by subject so that a zero represented the subject's best trial and a one represented the subject's worst trial, regardless of leg, with the relative differences between their performances maintained (TTS_{Scale} and DPSI_{Scale}). This was done because each subject inherently had different DPS. That is, no matter how many trials they performed, some subjects demonstrated better DPS than others. Rescaling the dependent variables removed this inherent difference between subject's making the effect of trial more apparent and allowed for more insight into each subject's improvement over trials toward their unique and theoretical best performance. The rolling means and standard deviation for TTS_{Scale} and DPSI_{Scale} were then computed separately by subject and leg using a window width of three and a slide of 1 (TTS_{Mean} and DPSI_{Mean} and TTS_{SD} and DPSI_{SD}, respectively). This resulted in the formation of 8 new clusters composed of 3 trials each where the cluster name is the center trial (e.g., cluster 2 =trials 1, 2, and 3; cluster 3 = trials, 2, 3, and 4;...cluster 9 = trials 8, 9, 10). This was done for several reasons. The trial-to-trial mean and standard deviation typically decreases across attempts of a motor control task indicating improvement in performance and consistency. In a realworld setting, a clinician would have a subject perform a series of familiarization trials followed by a series of measured trials and then average the measured trials together for further analyses. For example, DuPrey et al. (2016) also took the mean of three measured trials for statistical analyses. Therefore, this approach identified the cluster of three consecutive trials with the best mean performance and lowest standard deviation. Further, the rolling mean and standard deviation also removed some inherent noise in the performance over trials that may have masked underlying trends in the data. Finally, an indicator of optimal TTS and DPSI performance (TTS_{Score} and

DPSI_{Score}) was calculated by multiplying TTS_{Mean} and TTS_{SD} as well as DPSI_{Mean} and DPSI_{SD}. A lower TTS_{Score} and DPSI_{Score} represented the optimal combination of performance and standard deviation for a given cluster of three trials. The same series of mixed effects models described above was then repeated to examine the magnitude of change in TTS_{Mean} and DPSI_{Mean}, TTS_{SD} and DPSI_{SD}, and TTS_{Score} and DPSI_{Score} over clusters of three trials by biological sex and leg.

3.6.2. Purpose and Hypothesis 2

The primary purpose of this study was to evaluate the reliability of DPS, as measured by TTS and DPSI, following a BSLJL and assess the effect of increasing the number of BSLJL trials on reliability and the minimal detectable change (MDC). Based on previous DPS investigations (Byrne et al., 2021; Colby et al., 1999; Ebben et al., 2010; Flanagan et al., 2008; Fransz et al., 2016; Fransz et al., 2015; Wikstrom et al., 2005), it was hypothesized that three trials per leg and the resulting mean value would elicit "moderate" to "good" reliability, as defined by a reliability coefficient between 0.50 and 0.90 (Koo & Li, 2016).

Data collected during visits two, three, and four were used to assess this hypothesis. The independent categorical variables of this study were leg (e.g., dominant or non-dominant), session (e.g., CONTROL, FATIGUE₁, or FATIGUE₂), time (e.g., PRE or POST) and trial (e.g., 1, 2, or 3). The dependent continuous variables were TTS and DPSI. Statistics were completed using R v. 4.0.5 (R Core Team, 2019) and accompanying package "gtheory" v. 0.1.2 (Moore, 2016). Descriptive statistics including means, 95% confidence intervals, medians, quartiles, minimum (25th Percentile – (1.5 · Interquartile Range)), and maximum (75th Percentile + (1.5 · Interquartile Range)) as well as normal Q-Q plots and histograms were used to initially explore the data.

Generalizability theory was used to assess the reliability of the dependent variables (Brennan, 1992; Shavelson & Webb, 1991). Classical test theory assumes that an observed value can be decomposed into the subject's true value and an error term. Generalizability theory extends classical test theory by recognizing that the error term can be further decomposed into one or more sources of error. In the present study, the sources of error, which are called facets in generalizability theory, included the independent categorical variables session, time, leg, and trial as well as the object of measure, the subject. In a generalizability study, the random effect variance $(\hat{\sigma}^2)$ for each facet is estimated, which in effect partitions the error into sources. The highest order interaction facet is the residual error or left over $\hat{\sigma}^2$ that cannot be allocated to a specific source. For ideal reliability, the majority of $\hat{\sigma}^2$ should be allocated into the facet for the subject, indicating that the largest source of variation in the dependent variable is due to inherent differences between the subjects (e.g., between subject variance). Poor reliability exists if the majority of $\hat{\sigma}^2$ is allocated to other sources, especially the residual error term. The $\hat{\sigma}^2$ for the facets obtained from the generalizability are then used to conduct a decision study. A strength of a decision study is that an investigator can assess how reliability would be affected if distinct aspects of the study were changed, such as increasing or decreasing the number of trials used to obtain the mean value. Decision studies also provide a number of relative or "norm-referenced" reliability and absolute or "domain-referenced" reliability metrics.

A four facet, fully crossed design (session: time: leg: trial) was used to perform a generalizability study and estimate $\hat{\sigma}^2$ for thirty-one facets. Relative error variance (REV; $\sum \hat{\sigma}^2$ for all facets that interact with subject except the main facet for subject) and absolute error standard deviation (AEV; $\sum \hat{\sigma}^2$ for all facets except the main facet for subject) was computed along with the relative minimal detectable change (MDC_{Rel}; $\sqrt{REV} \cdot 1.96 \cdot \sqrt{2}$), absolute minimal detectable

change (MDC_{Ab}; $\sqrt{AEV} \cdot 1.96 \cdot \sqrt{2}$), generalizability coefficient ($\hat{\sigma}^2$ for the main facet for subject \cdot ($\hat{\sigma}^2$ for the main facet for subject facet + *REV*¹), and dependability coefficient ($\hat{\sigma}^2$ for the main facet for subject \cdot ($\hat{\sigma}^2$ for the main facet for subject facet + AEV)⁻¹). The generalizability and dependability coefficients are analogous to the interclass correlation coefficient (ICC) used in classical test theory, with greater coefficients representing greater reliability. Although there are no guidelines describing coefficient thresholds (e.g., "poor", "moderate", etc.) for the generalizability and dependability coefficients specifically, and even deducing ICC values into these qualitative ranges is very context specific, to aid in interpreting the coefficients presented here a value less than 0.50 was considered poor, 0.50 to 0.75 was considered moderate, 0.75 to 0.90 was considered good, and greater than 0.90 was considered excellent (Koo & Li, 2016). A specific aim of this paper is to determine the reliability of the dependent variables when different numbers of trials per leg are performed. Based on classical test theory and the assumption of random error, as additional trials are performed and averaged together, the subject's mean value form the measured trials and their true but unknown value get closer together and the error term decreases resulting in improved reliability. Decision studies were therefore completed by adjusting the number of days, sessions, and times for testing to one and varying the number of trials performed per leg (e.g., 1, 2, 3...10) and re-computing the reliability metrics. No probability or hypothesis testing is conducted with a generalizability or decision study.

3.6.3. Purpose and Hypothesis 3

The primary purpose of this study was to evaluate the relation between biological sex, fatigue, hip external rotation and abduction strength, and DPS as measured by TTS and DPSI. Based on previous investigations it was hypothesized that DPS would be compromised after completion of a short, high-intensity exercise bout (Bond et al., 2020; Shaw et al., 2008; Wikstrom

et al., 2004) and that subjects with greater hip strength will demonstrate superior DPS (Bandholm et al., 2011; Lephart et al., 2002; Neamatallah et al., 2020; Suzuki et al., 2015; Williams et al., 2016; Zazulak et al., 2005).

PRE and POST data from visits two, three, and four was used to address this purpose. The independent categorical variables of this study were biological sex (e.g., male or female), leg (e.g., dominant or non-dominant), session type (e.g., CONTROL or FATIGUE), and time (e.g., PRE or POST). The independent continuous variables of this study were relative peak force and torque for hip abduction and external rotation. The dependent continuous variables were TTS and DPSI. Statistics were completed using R v. 4.0.5 (R Core Team, 2019) and accompanying packages "lme4" v. 0.1.2 (Bates et al., 2022) and "emmeans" v. 1.7.2 (Lenth et al., 2022). Descriptive statistics including means, 95% confidence intervals, medians, quartiles, minimum $(25th Percentile - (1.5 \cdot Interquartile Range))$, and maximum $(75th Percentile + (1.5 \cdot Interquartile Range))$ as well as normal Q-Q plots and histograms were used to initially explore the data.

TTS and DPSI were assessed using separate linear mixed-effects models where a full factorial of session type (e.g., CONTROL and FATIGUE), time (e.g., PRE and POST), and biological sex (e.g., male and female) were entered as fixed-effects and each subject was assigned a random intercept. If appropriate, Bonferroni corrected post-hoc tests were performed to identify the source of the effect for any significant effects.

TTS and DPSI were then aggregate by subject for CONTROL, PRE FATIGUE, and POST FATIGUE, separately. The difference (PRE-POST) between each subject's aggregated TTS and DPSI from PRE to POST FATIGUE will be computed by subtracting their POST mean from PRE mean. Pearson correlations were then be used to assess the relation between hip abduction and external rotation force and torque and TTS and DPSI during CONTROL, POST FATIGUE, and PRE-POST FATIGUE. Significance was set to p < 0.05 for all statistical analyses.

4. EFFECT OF REPEATED BACKWARD SINGLE-LEGGED JUMP-LANDINGS ON DYNAMIC POSTURAL STABILITY: WITHIN-SESSION MOTOR LEARNING 4.1. Introduction

It is imperative to define within-session familiarization protocols for assessments of musculoskeletal injury risk to optimize assessment reliability and subsequent discriminatory capacity (Hopkins, 2000; Weir, 2005). The anterior cruciate ligament (ACL) is the most frequently injured knee ligament (Musahl & Karlsson, 2019). The incidence of ACL injury is highest for adolescents and young adults between 15 and 34 years of age (Renstrom et al., 2008), peaking at 16 to 18 years of age, with a high proportion of these injuries occurring during athletic or physical activity participation (Musahl & Karlsson, 2019). It has been estimated that over half of all ACL injuries are attributed to a non-contact mechanism, meaning the only appreciable force acting on the athlete immediately prior to and at the time of injury was gravity (Musahl & Karlsson, 2019). Frequent non-contact inciting events that result in ACL injury include suddenly decelerating or changing direction, with ACL injury specifically occurring during the eccentric or weight absorption phase of the movement (Krosshaug et al., 2007; Olsen et al., 2004; Walden et al., 2015). With this in mind, assessments of ACL injury risk predominately feature tasks that require single-legged neuromuscular eccentric control, such as a single-legged jump-landing.

Dynamic postural stability (DPS) is one indicator of single-legged neuromuscular eccentric control and is defined as the ability of a subject's neuromuscular system to obtain stability during a shift from a dynamic movement to a stationary position over the base of support (DuPrey et al., 2016; Liu & Heise, 2013; Ross & Guskiewicz, 2003; Wikstrom et al., 2005). Assessing DPS following a single-legged jump-landing requires the collection ground reaction forces (GRF), which can then be used to quantify DPS using one of many computational methodologies,

including time to stabilization (TTS) and the dynamic postural stability index (DPSI) (DuPrey et al., 2016; Liu & Heise, 2013; Ross & Guskiewicz, 2003; Wikstrom et al., 2005). DPS has been used to assess post-ACL reconstruction (ACLR) and ACL deficient subjects and demonstrated sufficient sensitivity to detect residual deficits in neuromuscular eccentric control (Colby et al., 1999; Webster & Gribble, 2010). Colby et al. (1999) assessed DPS following a forward single-legged jump-landing in adults 4 to 6 months post-ACLR, adults with ACL deficiency, and adults without a history of ACL injury. The authors found that DPS had the potential to distinguish between the injured and uninjured leg and suggested that DPS could be used to monitor progress during rehabilitation (Colby et al., 1999). Webster and Gribble (2010) assessed DPS following a forward single-legged jump-landing in female college athletes with a history of ACLR (mean 2.5 y post-ACLR) and age and sport matched athletes without a history of ACLR. The investigators demonstrated that the athletes with a history of ACLR demonstrated worse DPS compared to the healthy controls, potentially suggesting that deficits in neuromuscular eccentric control persist well after ACLR and presumably after the athlete returns to sport.

Poor DPS has been prospectively identified as a risk factor for non-contact ACL injury (DuPrey et al., 2016). DuPrey et al. (2016) assessed DPS following backward, forward, lateral, and medial single-legged jump-landings in collegiate student-athletes. DPS was quantified as TTS, defined as the time between initial contact to when vertical ground reaction force (GRF_{Vert}) reached and remained with 5% of the athlete's body mass for the remainder of the trial (DuPrey et al., 2016). The authors revealed that student-athletes who subsequently sustained a non-contact ACL injury demonstrated a baseline TTS that was 50% longer following the backwards single-legged jump-landing (BSLJL) compared to their peers who did not subsequently sustain an ACL injury (DuPrey et al., 2016). Baseline TTS following the forward, lateral, and medial jump-landing were

not different between those that subsequently sustained a non-contact ACL injury and those that did not (DuPrey et al., 2016). It is unclear why only TTS following the BSLJL at baseline was associated with the odds of sustaining a subsequent ACL injury and TTS from the other directions did not, though it is possible that a BSLJL is more demanding task compared to the other directions and that this higher demand increased the variance in TTS between those with sufficient and insufficient DPS capacity, which is reflective in its ability to discriminate ACL injury risk levels. Further, sufficiently challenging tasks better elucidate deficiencies if present in a particular athlete. Nevertheless, given the rarity of prospectively identified relations between an injury risk assessment and occurrence of injury, further evaluation of DPS, and TTS in particular, following a BSLJL is crucial.

Even though the assessment of TTS may be considered lab-based, the simplicity and intuitive nature in contrast to more complicated measures, such as joint moments that also require kinematic data, as well as the increasing accessibility to devices that measure ground reaction force makes TTS highly appealing for widespread implementation. Within a testing session, subjects tend to improve their performance and consistency of performance with repeated attempts of a motor control task (Magill & Anderson, 2010), which is measured by decreasing (improving) TTS and decreasing trial-to-trial standard deviation in TTS, respectively. Greater improvement in performance and trial-to-trial consistency is typically observed if the motor control task is novel to the subject. Familiarization trials, which are typically unmeasured, are usually performed at the beginning of the testing session to reduce, but likely not eliminate, this motor learning effect prior to performing the measured trials which are subsequently used to classify injury risk. For example, DuPrey et al. (2016) had student-athletes complete three familiarization trials on each leg from each jump direction (24 total familiarization trials) prior to completing three measured trials on

each leg from each jump direction (24 total measured trials). It is possible that this number of familiarization trials may have adequately reduced the motor learning effect, especially considering transfer learning likely occurred from one leg or jump direction to another (Magill & Anderson, 2010). Nevertheless, the number of familiarization trials needed at the beginning of a testing session to sufficiently reduce the motor learning effect prior to performing measured trials is unclear, especially if a clinician were to only utilize one single-legged jump-landing direction, such as the BSLJL identified by DuPrey et al. (2016). The number of familiarization trials needed prior to measuring DPS has been assessed using a double-limb forward jump from a box with a single-legged jump-landing and 3 familiarization trials were recommended (VanMeter, 2007), but the number of familiarization trails has not been assessed for a BSLJL with TTS calculated in a manner similar to DuPrey et al. (2016). Thus, further investigation regarding within-session familiarization to a BSLJL and its effect on TTS is imperative and timely if this assessment of injury risk is to be implemented on a large scale as an indicator of ACL injury risk.

The primary purpose of this study was to evaluate the effect of repeated BSLJL trials alternating legs on DPS, as measured by TTS and DPSI, to identify the number of familiarization trials required to sufficiently reduce the motor learning effect. The secondary purpose of this study was to assess the effect biological sex on BSLJL motor learning as measured by DPS within a single testing session. Based on previous single-legged motor control task investigations including those assessing DPS following a single-legged jump-landing (Dallinga et al., 2016; Ebben et al., 2010; Gribble et al., 2007; Hertel et al., 2000; Keklicek et al., 2019; Lephart et al., 2002; Magill & Anderson, 2010; Munro & Herrington, 2010; VanMeter, 2007; Wikstrom et al., 2006), it was hypothesized that three trials per leg would be needed to reduce the within-session learning effect and there would be no difference in motor learning between males and females.

4.2. Methods

4.2.1. Research Design

This study consisted of a single visit where subjects completed 10 BSLJL trials per leg with no prior familiarization trials to assess the effect of repeated BSLJL trials on TTS and DPSI and determine the number of familiarization trials needed to sufficiently reduce the motor learning effect.

4.2.2. Subjects

Ten and seventeen recreationally active males and females, respectively, $(24.0 \pm 2.8 \text{ y}, 1.73 \pm 0.08 \text{ m}, 75. \pm 14.0 \text{ kg})$ between 12 and 30 years of age completed this study. This demographic was chosen as it represents the highest proportion of subjects who experience ACL injuries. Subjects with a prior ligamentous, bony, or other soft tissue operative procedures involving the lower extremity, an orthopedic issue exacerbated by exercise, acute fracture, tumor, or infection, unfavorable cardiovascular responses to exercise, a neurological condition that effects the activation of skeletal muscle or balance, and diabetes were excluded from this study. Additionally, active smokers or those that have smoked in the past 6 months, pregnant females, and cognitively impaired adults were also excluded from this study. The Sanford Health Institutional Review Board approved all aspects of this study (approval number: 1009). Subjects were informed of the study's protocol, benefits, and risks before providing their informed, written, voluntary consent. None of the subjects were less than 18 years of age.

4.2.3. Procedures

The subject's dominant leg was recorded as the leg in which the subject indicated they would kick a soccer ball with. The subject then completed a guided 10-minute warm up consisting of light aerobic exercise, dynamic stretching, and plyometrics. The subject then had the BSLJL,

described in detail below, demonstrated to them by the investigator and could ask the investigator questions about the task for clarification. Prior to performing the first BSLJL trial, the subject stepped onto the force plate and remained as motionless as possible. Ground reaction force data was sampled for 10 seconds to obtain the subject's body weight (BW; N). The subject then performed ten trials per leg (20 trials total) by alternating between the dominant and non-dominant leg to avoid an acute fatigue effect with 15 seconds of rest between trials. The leg (e.g., dominant or non-dominant) that performed the first trial was randomized to control for an order effect. Since the purpose of this research study was to examine the within session learning effects of repeatedly performing this task on TTS and DPSI, no unmeasured or measured familiarization trials were performed prior to these trials.

4.2.4. Backwards Single-Legged Jump-Landing Task

The BSLJL used in this study was identical to the protocol used and described by DuPrey et al. (2016), which was adopted from Liu and Heise (2013). A 0.05 m tall hurdle was placed parallel with the edge of the force plate to standardize the minimal foot clearance off the ground required to complete the task. The subject started the task by standing on two feet directly next to a force plate with their back facing the force plate and their hands on their hips. An investigator gave the subject an audible "three, two, one, go" command. The subject then lifted the non-test leg off the ground and jumped off the test leg backwards over the hurdle and onto the force plate. The subject was instructed to land on the force plate on the test leg with their eyes focused forward and their hands on their hips, stabilize as quickly as possible, and remain motionless until the investigator indicated the trial was over. Trials were performed barefoot to minimize the stability provide by a shoe. Following the initial contact, the subject was permitted to hop or shuffle on their test leg to stabilize if their test leg foot did not contact any surrounding surface besides the force plate. Trials where the subject removed their hands from their hips upon landing, touched their non-test leg or any other body part to the ground, or contacted their test leg to any surrounding surface besides the force plate were considered failed trials. Failed trials were documented but were not repeated as a failed trial would be considered additional familiarization.

4.2.5. Data Processing

Data was processed using a custom written MATLAB program (R2021a, MathWorks, Natick, MA, USA). Raw GRF_{Vert} and horizontal ground reaction forces (GRF_{AP} and GRF_{ML}) were filtered post-hoc using a second order 12 Hz low-pass Butterworth filter (Ross et al., 2005; Webster & Gribble, 2010). All further use of GRF data utilizes filtered GRF_{Vert}, GRF_{AP}, and GRF_{ML}. It should be noted that various filters have been used to process GRF data and it is recognized that different order and frequency low pass filters will elicit different DPS metrics (Fransz et al., 2015). The subject's body mass (N·9.81⁻¹; kg) was calculated as the mean GRF_{Vert} from the ten second trial performed at the beginning of the visit.

Initial contact was defined as the instant GRF_{Vert} first exceeded 20 N. GRF data was cropped into 3- and 5-second post-initial contact time frames (GRF_{Vert3} , GRF_{AP3} , and GRF_{ML3} and GRF_{Vert5} , GRF_{AP5} , and GRF_{ML5} , respectively). Time to stabilization (TTS) was calculated using GRF_{Vert5} as the length of time in seconds (Δ time; s) required for GRF_{Vert5} to reach and then remain between 95% and 105% of the subject's body weight (GRF_{Vert5} ·body weight in N⁻¹; %) for the remainder of the trial (DuPrey et al., 2016). Dynamic postural stability indices were calculated using the methodology described by Wikstrom et al. (2005) and modified by (Dallinga et al., 2016; Wikstrom et al., 2010) using GRF_{Vert3} , GRF_{ML3} , and GRF_{AP3} . Stability indices for GRF_{ML} (MLSI), GRF_{AP} (APSI), and GRF_{Vert} (VSI) reflect the average magnitude of fluctuation (standard deviation) of GRF_{Vert3} , GRF_{ML3} , and GRF_{AP3} vectors around 0 N for MLSI and APSI and the subject's body weight in N for VSI. DPSI represents a composite score of MLSI, APSI, and VSI. These indices were calculated using the following equations (Dallinga et al., 2016; Wikstrom et al., 2010) where body weight is the subject's body weight in N and samples is the number of GRF data points included (e.g., 3,000 for a 3 second post-initial contact time frame if recorded at 1 kHz as done here):

$$Medial - Lateral Stability Index = \sqrt{\sum ((0 - GRF_{ML}) \cdot Body Weight^{-1})^{2} \cdot Samples^{-1}}$$

$$Anterior - Posterior Stability Index = \sqrt{\sum ((0 - GRF_{AP}) \cdot Body Weight^{-1})^{2} \cdot Samples^{-1}}$$

$$Vertical Stability Index = \sqrt{\sum ((GRF_{Vert} - Body Weight) \cdot Body Weight^{-1})^{2} \cdot Samples^{-1}}$$

 $Dynamic \ Postural \ Stability \ Index = \sqrt{((\sum (0 - Fx)^2 + \sum (0 - Fy)^2 + \sum (Body \ Weight - Fz)^2) \cdot Body \ Weight^{-1})) \cdot Samples^{-1}}$

Data for all calculated variables were entered into a data matrix. Because "failed" trials were not repeated, cells corresponding to them were left blank. Cells corresponding to TTS were also left blank for trials in which the stability threshold, defined as reaching and remaining between 95% and 105% of the subject's body weight for the remainder of the trial, was not achieved within the 5-second post-initial contact period.

4.2.6. Statistical Analyses

The independent categorical variables of this study were leg (e.g., dominant or nondominant), sex (e.g., male and female), and trial (e.g., 1, 2,...10). The dependent continuous variables were TTS and DPSI. Statistics were completed using R v. 4.0.5 (R Core Team, 2019) and accompanying packages "lme4" v. 1.1-28 (Bates et al., 2022) and "emmeans" v. 1.7.2 (Lenth et al., 2022). Descriptive statistics including means, 95% confidence intervals, medians, quartiles, minimum (25th Percentile – (1.5 · Interquartile Range)), and maximum (75th Percentile + (1.5 · Interquartile Range)) as well as normal Q-Q plots and histograms were used to initially explore the data. A series of linear mixed effects models were then conducted to examine the magnitude of change in TTS and DPSI over trials by biological sex and leg. In these models, a full factorial of trial, biological sex, and leg were entered as fixed effects and each subject was assigned a random intercept. Estimated marginal means were then computed at each level of the fixed effects. If appropriate, Bonferroni corrected post-hoc comparisons were used to assess the trial-to-trial change (e.g., trial 2 - trial 1, 3-2...10-9) in the dependent variables by biological sex and leg. Additionally, the linear and quadratic contrasts for each biological sex and leg were also computed to evaluate underlying linear and quadratic changes in the dependent variables over trials. Significance was set at p < .05.

TTS and DPSI were then separately rescaled by subject so that a zero represented the subject's best trial and a one represented the subject's worst trial, regardless of leg, with the relative differences between their performances maintained (TTS_{Scale} and DPSI_{Scale}). This was done because each subject inherently had different DPS. That is, no matter how many trials they performed, some subjects demonstrated better DPS than others. Rescaling the dependent variables removed this inherent difference between subject's making the effects of trial more apparent and allowed for more insight into each subject's improvement over trials toward their unique and theoretical best performance. The rolling mean and standard deviation for TTS_{Scale} and DPSI_{Scale} were then computed separately by subject and leg using a window width of three and a slide of 1 (TTS_{Mean} and DPSI_{Mean} and TTS_{SD} and DPSI_{SD}, respectively). This resulted in the formation of 8 new clusters composed of 3 trials each where the cluster name is the center trial (e.g., cluster 2 = trials 1, 2, and 3; cluster 3 = trials, 2, 3, and 4;...cluster 9 = trials 8, 9, 10). This was done for several reasons. The trial-to-trial mean and standard deviation typically decrease across attempts of a motor control task indicating improvement in performance and consistency. In a real-

world setting, a clinician would have a subject perform a series of familiarization trials followed by a series of measured trials and then average the measured trials together for further analyses. This approach identified the cluster of three consecutive trials with the best mean performance and lowest standard deviation. For example, DuPrey et al. (2016) also took the mean of three measured trials for statistical analyses. Further, the rolling mean and standard deviation removed some inherent noise in the performance over trials that may have masked underlying trends in the data. Finally, an indicator of optimal TTS and DPSI performance (TTS_{Score} and DPSI_{Score}) were calculated by multiplying TTS_{Mean} and TTS_{SD} as well as DPSI_{Mean} and DPSI_{SD}. A lower TTS_{Score} and DPSI_{Score} represented the optimal combination of performance and standard deviation for a given cluster of three trials. The same series of mixed effects models described above were then repeated to examine the magnitude of change in TTS_{Mean} and DPSI_{Mean}, TTS_{SD} and DPSI_{SD}, and TTS_{Score} and DPSI_{Score} over clusters of three trials by biological sex and leg.

4.3. Results

For TTS and DPSI, histogram and Q-Q plots by leg and biological sex are presented in Figure 5, spaghetti plots illustrating the trial-to-trial change by subject, leg, and biological sex are presented in Figure 6, and a boxplot demonstrating the median, quartiles, minimum, and maximum for each trial by leg and biological sex is presented in Figure 7. There was a total of fifty-three failed trials (10%). Twenty-one failed trials occurred on the first trial on either the dominant or non-dominant leg, eight failed trials occurred on the second trial on either the dominant or non-dominant leg, and then less than 6 failed trials occurred on the remaining trials on either the dominant or non-dominant leg.
Histogram and Q-Q plot of time to stabilization (TTS) and dynamic postural stability index (DPSI) over ten trials of a backwards single-legged jump-landing on the dominant and non-dominant leg for males (blue) and females (pink)



Spaghetti plot of time to stabilization (TTS) and dynamic postural stability index (DPSI) over ten trials of a backwards single-legged jump-landing on the dominant and non-dominant leg for males (blue) and females (pink)



Boxplot of time to stabilization (TTS) and dynamic postural stability index (DPSI) over ten trials of a backwards single-legged jump-landing on the dominant and non-dominant leg for males (blue) and females (pink)



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For TTS and DPSI, means and 95% confidence intervals by leg and biological sex are presented in Figure 8. None of the fixed effects in the mixed effects model assessing the magnitude of change in TTS over trials were significant (Table 2). Conversely, biological sex was the only significant fixed effect in the mixed effects model for DPSI, suggesting that females had lower (better) DPSI than males. Because the fixed effects of trial were not significant, no trial-to-trial post-hoc comparisons were conducted for TTS or DPSI. Linear and quadratic contrasts for trial evaluating underlying linear and quadratic change in TTS and DPSI revealed that female's TTS on the non-dominant leg had a pronounced linear decrease across trials (Table 3). No other significant linear and quadratic contrasts were identified for TTS or DPSI.

Means and 95% confidence intervals of time to stabilization (TTS) and dynamic postural stability index (DPSI) over ten trials of a backwards single-legged jump-landing on the dominant and non-dominant leg for males (blue) and females (pink)



Analysis of variance table from the mixed effects model for the independent categorical variables of trial, leg, and biological sex for time to stabilization (TTS) and dynamic postural stability index (DPSI) over ten trials of a backwards single-legged jump-landing

Factor	Number of Parameters	TTS		DPSI	
		F-Value	P-value	F-value	P-value
Trial	9	1.40	0.18	0.90	0.52
Biological Sex	1	0.01	0.91	6.63	0.01*
Leg	1	0.11	0.74	1.56	0.21
$Trial \times Biological \ Sex$	9	0.48	0.88	0.81	0.59
Trial \times Leg	9	0.81	0.60	0.57	0.82
$\text{Leg} \times \text{Biological Sex}$	1	0.01	0.89	2.41	0.12
Trial × Biological Sex × Leg	9	0.79	0.62	0.34	0.95

Linear and quadratic contrasts across trials for time to stabilization (TTS) and dynamic postural stability index (DPSI) following a backwards single-legged jump-landing on the dominant and non-dominant leg for males and females

	TTS				DPSI	
Contrast	Estimate	T-value	P-value	Estimate	T-value	P-value
		Male: Domi	nant			
Linear	-6.56	-1.21	0.22	-2.00	-1.21	0.22
Quadratic	0.81	0.24	0.81	0.86	0.83	0.40
]	Female: Don	ninant			
Linear	-6.61	-1.44	0.14	-0.79	-0.57	0.56
Quadratic	1.75	0.61	0.54	0.63	0.72	0.46
	Ν	Iale: Non-do	minant			
Linear	-7.50	-1.43	0.15	-1.45	-0.91	0.36
Quadratic	5.96	1.80	0.07	0.68	0.68	0.49
Female: Non-dominant						
Linear	-11.55	-2.50	0.01*	-1.30	-0.92	0.35
Quadratic	-4.42	-1.53	0.12	0.08	0.09	0.92

For TTS_{Mean} and DPSI_{Mean}, means with 95% confidence intervals by leg and biological sex are presented in Figure 9. For the mixed effect model assessing the magnitude of change in TTS_{Mean} over clusters of three trials, the fixed effect of trial was significant (Table 4); however, none of the Bonferroni corrected post-hoc comparisons assessing the difference from one cluster to the next consecutive clusters were significant. For the mixed effect of leg was significant suggesting that subjects' non-dominant leg had lower (better) DPSI compared to the dominant leg (Table 5). Linear and quadratic contrasts for trial evaluating underlying linear and quadratic change in TTS_{Mean} on the dominant and female's TTS_{Mean} on the dominant leg had pronounced linear decreases across clusters. Further, female's DPSI_{Mean} on the non-dominant leg also had a pronounced linear decrease across clusters of three trials.

Means and 95% confidence intervals of time to stabilization scaled (TTSMean) and dynamic postural stability index scaled (DPSIMean) over clusters of three trials of a backwards single-legged jump-landing on the dominant and non-dominant leg for males (blue) and females (pink)



Analysis of variance table from the mixed effects model for the independent categorical variables of cluster, leg, and biological sex for time to stabilization (TTS_{Mean}) and dynamic postural stability index $(DPSI_{Mean})$ over clusters of three trials of a backwards single-legged jump-landing

Factor	Number of Parameters	TTS		DPSI	
		F- Value	P- value	F- value	P- value
Cluster	9	2.37	0.02*	1.42	0.19
Biological Sex	1	2.24	0.14	0.29	0.59
Leg	1	2.14	0.14	5.48	0.01*
Cluster \times Biological Sex	9	0.38	0.91	0.24	0.97
Cluster \times Leg	9	0.88	0.51	1.25	0.27
$Leg \times Biological \ Sex$	1	0.03	0.85	1.79	0.18
Cluster × Biological Sex × Leg	9	0.22	0.98	0.18	0.98

Linear and quadratic contrasts across clusters of three trials for time to stabilization (TTS_{Mean}) and dynamic postural stability index $(DPSI_{Mean})$ following a backwards single-legged jumplanding on the dominant and non-dominant leg for males and females

		TTS			DPSI	
Contrast	Estimate	T-value	P-value	Estimate	T-value	P-value
		Male: Domi	nant			
Linear	0.35	-0.47	0.63	-0.44	-0.59	0.55
Quadratic	0.31	0.42	0.66	0.70	0.95	0.34
		Female: Dom	ninant			
Linear	-1.57	-2.45	0.01*	-0.75	-1.17	0.24
Quadratic	0.47	0.74	0.45	0.80	1.25	0.21
	Ν	Iale: Non-do	minant			
Linear	-1.59	-2.26	0.02*	-0.85	-1.19	0.23
Quadratic	0.45	0.66	0.50	0.33	0.46	0.63
Female: Non-dominant						
Linear	-1.98	-2.91	0.003*	-1.83	-2.65	0.008*
Quadratic	-0.67	-1.01	0.31	-0.21	-0.32	0.74

For TTS_{SD} and DPSI_{SD}, means with 95% confidence intervals by leg and biological sex are presented in Figure 10. For the mixed effects model assessing the magnitude of change in TTS_{SD} over clusters of three trials, the effect of biological sex was significant suggesting that males had better TTS consistency than females (Table 6). No significant effects were identified in the mixed effects model assessing the magnitude of change in DPSI_{SD} over clusters of three trials. Because the fixed effects of cluster were not significant, no cluster-to-cluster post-hoc comparisons were conducted for TTS_{SD} and DPSI_{SD}. Linear and quadratic contrasts for cluster evaluating underlying linear and quadratic change in TTS_{SD} and DPSI_{SD} revealed that females TTS_{SD} on the dominant leg had a pronounced linear decrease (improvement) across clusters of three trials (Table 7). Males DPSI_{SD} on the non-dominant leg had a pronounced quadratic trend across clusters of three trials whereas females DPSI_{SD} on the non-dominant leg had a pronounced linear decrease (improvement) across clusters of three trials.

Means and 95% confidence intervals of time to stabilization scaled (TTS_{SD}) and dynamic postural stability index scaled (DPSI_{SD}) over clusters of three trials of a backwards single-legged jump-landing on the dominant and non-dominant leg for males (blue) and female (pink)



Analysis of variance table from the mixed effects model for the independent categorical variables of cluster, leg, and biological sex for time to stabilization (TTS_{SD}) and dynamic postural stability index $(DPSI_{SD})$ over clusters of three trials of a backwards single-legged jump-landing

Factor	Number of Parameters	TTS		DPSI	
		F- Value	P- value	F- value	P- value
Cluster	9	0.90	0.50	1.02	0.41
Biological Sex	1	4.49	0.04*	0.13	0.71
Leg	1	0.00	0.96	1.60	0.20
Cluster \times Biological Sex	9	0.60	0.75	1.13	0.34
Cluster \times Leg	9	1.02	0.41	0.46	0.86
$\text{Leg} \times \text{Biological Sex}$	1	0.79	0.37	0.81	0.36
Cluster \times Biological Sex \times Leg	9	0.92	0.48	1.79	0.08

Linear and quadratic contrasts across clusters of three trials for time to stabilization (TTS_{SD}) and dynamic postural stability index $(DPSI_{SD})$ following a backwards single-legged jumplanding on the dominant and non-dominant leg for males and females

	TTS				DPSI	
Contrast	Estimate	T-value	P-value	Estimate	T-value	P-value
		Male: Domi	nant			
Linear	0.97	1.37	0.16	0.40	0.69	0.48
Quadratic	0.04	0.07	0.94	0.64	1.14	0.25
		Female: Dom	ninant			
Linear	-1.21	-2.03	0.04*	-0.79	-1.63	0.10
Quadratic	-0.40	-0.67	0.50	-0.21	-0.44	0.65
	Ν	Iale: Non-do	minant			
Linear	-1.20	-1.80	0.07	0.13	0.25	0.79
Quadratic	-0.78	-1.19	0.23	-1.07	-2.01	0.04*
Female: Non-dominant						
Linear	-0.90	-1.40	0.16	-1.04	-1.99	0.04*
Quadratic	-0.47	-0.75	0.45	0.71	1.39	0.16

Finally, for TTS_{Score} and $DPSI_{Score}$, means with 95% confidence interval by leg and biological sex are presented in Figure 11. For the mixed effects model assessing the magnitude of change in TTS_{Score} over clusters of three trials, the effect of biological sex was significant suggesting that males had a lower (better) TTS_{Score} than females (Table 8). For the mixed effects model assessing the magnitude of change in $DPSI_{Score}$ over clusters of three trials the effect of leg was significant indicating that the non-dominant leg had a lower (better) score than the dominant leg. Linear and contrasts for cluster evaluating the underlying linear and quadratic change in TTS_{Score} and $DPSI_{Score}$ revealed that male non-dominant and female non-dominant and dominant legs had a pronounced linear decrease across clusters of three trials (Table 9).

Time to stabilization score (TTS_{Score}) and dynamic postural stability index scaled ($DPSI_{Score}$) representing optimal performance for clusters of three trials of a backwards single-legged jumplanding on the dominant and non-dominant leg for males (blue) and female (pink)



Analysis of variance table from the mixed effects model for the independent categorical variables of cluster, leg, and biological sex for time to stabilization (TTS_{Score}) and dynamic postural stability index ($DPSI_{Score}$) over clusters of three trials of a backwards single-legged jump-landing

Factor	Number of Parameters	TTS		DPSI	
		F- Value	P- value	F- value	P- value
Cluster	9	1.10	0.35	1.43	0.19
Biological Sex	1	6.96	0.01*	0.04	0.83
Leg	1	0.32	0.57	4.34	0.03*
Cluster \times Biological Sex	9	0.42	0.88	1.00	0.42
Cluster \times Leg	9	1.11	0.35	0.70	0.66
$Leg \times Biological \ Sex$	1	0.52	0.46	2.19	0.13
Cluster \times Biological Sex \times Leg	9	0.30	0.95	1.13	0.34

Linear and quadratic contrasts across clusters of three trials for time to stabilization (TTS_{SD}) and dynamic postural stability index $(DPSI_{SD})$ following a backwards single-legged jumplanding on the dominant and non-dominant leg for males and females

	TTS				DPSI	
Contrast	Estimate	T-value	P-value	Estimate	T-value	P-value
		Male: Domi	nant			
Linear	0.27	0.74	0.45	0.12	0.36	0.71
Quadratic	0.09	0.27	0.78	0.62	1.92	0.05
]	Female: Dom	ninant			
Linear	-0.54	-1.73	0.08	-0.52	-1.87	0.06
Quadratic	-0.04	-0.14	0.88	0.07	0.26	0.78
	Ν	Iale: Non-do	minant			
Linear	-0.71	-2.02	0.04*	-0.01	-0.03	0.96
Quadratic	-0.24	-0.69	0.48	-0.41	-1.34	0.18
Female: Non-dominant						
Linear	-0.79	-2.34	0.01*	-0.73	-2.43	0.01*
Quadratic	-0.14	-0.43	0.66	0.23	0.80	0.42

4.4. Discussion

If DPS following a BSLJL is to be implemented as a measure of ACL injury risk, a greater understanding of the number of familiarization trials needed at the beginning of a testing session to sufficiently reduce the within-session motor learning effect is desirable as this will lead to the most reliable and therefore discriminative indicator of risk. Based on previous single-legged motor control task investigations including those assessing DPS following a single-legged jump-landing (Dallinga et al., 2016; Ebben et al., 2010; Gribble et al., 2007; Hertel et al., 2000; Keklicek et al., 2019; Lephart et al., 2002; Magill & Anderson, 2010; Munro & Herrington, 2010; VanMeter, 2007; Wikstrom et al., 2006), it was hypothesized that three trials per leg would be needed to reduce the within-session learning effect and there would be no difference in motor learning between males and females. Findings in relation to this hypothesis are equivocal.

Motor learning represents the change in the capability of a subject to perform a motor control task (e.g., BSLJL with optimal DPS) as a result of practice or experience within and between sessions (Magill & Anderson, 2010). Two characteristics of motor learning are improved performance and trial-to-trial consistency (Magill & Anderson, 2010), and these characteristics are especially apparent if the motor control task is novel to the subject. Familiarization trials are typically completed at the beginning of a testing session prior to measured trials so that the measures of motor control task performance used in subsequent analyses, such as those to stratify ACL injury risk, are free of the effects of motor learning that could misrepresent a subject. In a statistical sense, familiarization trials prior to measurement of motor control task performance shifts a proportion of the variance in performance from between trials within a subject to between subjects. However, there are diminishing returns in motor control task improvement with repeated trials, and after an excessive number of trials some subjects may experience a degree of fatigue or

attention loss that results in worsening performance (Magill & Anderson, 2010). Identifying this inflection point where motor control task performance or consistency no longer appreciably improves or is satisfactory before the onset of fatigue or attention loss is important as this leads to the most efficiency, reliable and discriminative injury risk screen.

In the present study, there is no evidence of a difference in TTS between legs and biological sexes averaged over trials. However, there is a difference in DPSI between biological sexes averaged over legs and trials. Females have a lower (better) DPSI than males, which was also demonstrated by Dallinga et al. (2016) who used the same DPSI computational methodology used here. It should be noted that other investigations that have used DPSI may not have corrected for body weight (Wikstrom et al., 2005), which could lead to erroneous findings, especially when comparing males and females who very likely have different mean body weights. Further, DPSI uncorrected for the subject's body weight could not be used to evaluate DPS over time within a subject if the subject's body weight increases or decrease, as would be expected with maturation and growth during adolescence. Although, TTS can be used to directly compare DPS between subjects and within a subject over time regardless of body weight.

The greatest number of failed trials, which were considered trials where the subject's hands came off their hips, the subject hopped or shuffled off the force plate, or any part of the subject's body touched the floor besides their test leg foot, occurred on either the first or second trial on the dominant or non-dominant leg. Therefore, it is recommended that at least two familiarization trials are performed per leg of the BSLJL to increases the odds of successful measured trials; although, additional trials are needed to sufficiently reduce the motor learning effect as discussed further. On average, males displayed the lowest (best) TTS on the dominant and non-dominant leg on trials 10 and 7, respectively, and females on trials 4 and 8, respectively. For DPSI, on average males displayed their lowest (best) performance on the dominant and non-dominant leg on trials 5 and 5, respectively, and females on trials 5 and 1, respectively. This may superficially suggest that more familiarization trials are needed to remove the motor learning effect prior to measurement of TTS compared to DPSI and that a sufficient number of familiarization trials potentially lies between 4 and 10 per leg. However, subjects randomly exhibit fluctuations in TTS and DPSI from trial-totrial, with TTS appearing to display larger within subject trial-to-trial fluctuations compared to DPSI. Additionally, both TTS and DPSI appear to be positively skewed, suggesting that for most trials TTS and DPSI are relatively low (better) with less frequent higher (worse) values that could be considered "outliers." Despite discovering a prominent linear decrease (improvement) in female's TTS on the non-dominant leg across trials, these random fluctuations in performance make it challenging to identify the underlying motor learning characteristics across trials within a specific subject, particularly in the presence of high between subject variance due to differences in the subject's innate DPS and within subject trial-to-trial variance (consistency). This was the impetus for rescaling TTS and DPSI and computing the rolling mean and standard deviation and reporting data for clusters of three trials. The cluster with the lowest (best) mean performance and/or lowest (best) standard deviation would therefore symbolize the three trials that an investigator would want to use as the measured trials, and all preceding trials would be considered familiarization trials.

For TTS_{Mean} , on average males displayed the lowest (best) performance on the dominant and non-dominant leg on clusters 3 and 8, respectively, and females on clusters 9 and 9, respectively. For DPSI_{Mean}, on average males displayed their lowest (best) performance on the dominant and non-dominant leg on clusters 4 and 7, respectively, and females on clusters 4 and 8, respectively. This indicates that the dominant leg reaches the cluster with its best performance after less trials than the non-dominant leg, potentially suggesting differences in the motor learning rate between legs. These findings also may indicate that males reach the cluster with their best performance after slightly less trials than females, also suggesting differences in the motor learning between biological sexes. For TTS_{Mean}, there was an effect of cluster, although none of the posthoc tests evaluating cluster-to-cluster changes in TTS_{Mean} were significant, and there were strongly pronounced linear decreases (improvements) in TTS_{Mean} for male's dominant and female's dominant and non-dominant legs, highlighting significant motor learning occurs across trials. For DPSI_{Mean}, the effect of leg was significant, suggesting that the non-dominant leg on average had lower (better) DPSI than the dominant leg, which is unexpected, and there was a marked linear decrease (improvement) in DPSI_{Mean} for female's non-dominant leg.

For TTS_{SD}, on average males displayed the lowest (best) performance on the dominant and non-dominant leg on clusters 3 and 8, respectively, and females on clusters 9 and 9, respectively. For DPSI_{SD}, on average males displayed their lowest (best) performance on the dominant and nondominant leg on clusters 4 and 4, respectively, and females on clusters 9 and 6, respectively. For TTS_{SD}, these are the same clusters that produced the lowest (best) TTS_{Mean}, which is predictable given a cluster with the lowest mean value likely contains 3 trials with similar (low standard deviation) low values themselves, but for DPSI_{SD} these clusters are slightly different than the clusters that produced the best DPSI_{Mean}, indicating that the cluster with the best mean performance and lowest trial-to-trial standard deviation do not always coincide. For TTS_{SD}, there was an effect of biological sex as males had a lower TTS_{SD} compared to females indicating that males have inherently lower trial-to-trial standard deviation, which was also unexpected. Female's dominant limb also displayed a distinct linear decrease in TTS_{SD} across clusters. These findings, combined with males reaching the cluster with their best performance after less trials than females when measured by TTS, potentially further corroborates differences in the motor learning between biological sexes. For DPSI_{SD}, male's non-dominant leg displayed a pronounced quadratic trend across clusters as DPSI_{SD} appears to initially get higher (worse) and then get lower (better), whereas female's non-dominant leg had a pronounced linear decrease (improvement) across clusters.

TTS_{Score} and DPSI_{Score}, which are indices that represent the cluster with the optimal combination of performance and standard deviation, were computed to attain final recommendations for the number of familiarization trials required to sufficiently reduce, but likely not eliminate, the motor learning effect. The lowest (best) TTS_{Score} for males on the dominant and non-dominant leg were observed for clusters three, and nine, respectively, and for females on clusters 8 and 9, respectively. The lowest (best) DPSIscore for males on the dominant and nondominant leg were observed for clusters 4 and 9, respectively, and for females on clusters 8 and 9, respectively. For TTS_{Score}, there was an effect of biological sex indicating that males had lower (better) TTS_{Score} than females, similar to what was observed for TTS_{SD}, and male's and female's non-dominant leg both displayed marked linear decreases (improvements) in TTS_{Score} over clusters. For DPSI_{Score}, there was an effect of leg indicating that the non-dominant leg had a lower (better) DPSIscore than the dominant leg, similar to what was observed for DPSIMean, and females on the non-dominant leg displayed a prominent linear decrease (improvement) in DPSI_{Score} over clusters. Dominant leg for males may reach optimal conditions after just 1 or 2 familiarization trials on both legs, but the non-dominant leg for males and both legs for females may require six or more familiarization trials on both legs to sufficiently reduce the motor learning effect. Although, it is possible that appreciable motor learning continues beyond the 10th trial per leg, especially for females. Based on these collective findings, it is recommended that a minimum of six familiarization trials per leg are completed prior to measuring trials 7, 8, and 9, with the assumption that the mean of three trials will be used for analysis and injury risk stratification.

One unique aspect of this study was that failed trials were noted but not repeated. Other studies, including DuPrey et al. (2016), repeat failed trials. The likely reason for this is to ensure that each subject has an identical number of trials available that can be either averaged together and used for analysis or entered into a number of statistical analyses that require complete observations (e.g., ANOVA). Although this practice is appreciated, investigators should be aware that motor learning still occurs on failed trials. Subjects who perform additional trials due to failures may display better DPS simply because they had more familiarization trials, which is what they amount to since they are not measured even if they fall between trials that are measured. For example, a subject who failed several trials but has an identical mean performance compared to a subject who failed no trials will have their injury risk stratified similarly, but it may be reasonable to speculate that the subject who failed several trials is actually more at-risk and that the reason for the failed trials is in some way related to a neuromuscular eccentric control deficit. Nevertheless, the inability to meaningfully incorporate failed trials into the analyses other than repeating failed trials or skipping failed trials and leaving the corresponding data value blank for analysis represents a statistical limitation that extends beyond the present study. Finally, this study represented the within session motor learning effects for a motor control task that was novel to the subject. If the subjects already had experience with this motor control task, it is possible that the within session motor learning effects would be different and that the subject may reach an optimal combination of lowest (best) performance and lowest (best) trial-to-trial standard deviation after less familiarization trials. This would be particularly important to evaluate if TTS and DPSI following a BSLJL were to be used as a longitudinal indicator of ACL injury risk.

There are no studies that used TTS and DPSI to quantify DPS following a BSLJL to directly compare the within-session motor learning findings of this study to, but there are studies that investigated motor learning across trials for other single-legged motor control tasks. Perhaps in the most similar study to this one, VanMeter (2007) assessed the number of familiarization trials at the beginning of a session needed prior to measuring DPS during a forward single-legged jumplanding and concluded that 3 familiarization trials were needed. Webster and Gribble (2010) assessed DPS in female collegiate student-athletes with and without a history of ACL injury and reported that their subjects needed 2 to 4 familiarization trials at the beginning of the testing session before feeling subjectively comfortable enough to begin measured trials. Studies that utilized the star excursion balance test to evaluate single-legged postural stability motor learning have suggested that four to seven trials within a session are needed to remove the motor learning effect (Gribble et al., 2007; Hertel et al., 2000; Munro & Herrington, 2010). Another investigation noted three separate sessions of familiarization to the star excursion test were needed to remove the motor learning effect with seven trials in each session (Keklicek et al., 2019), which indicates that between session or day retention and continued practice is an important component of motor learning.

There are several limitations of this study and possibilities for future research. Although this study used young adults, the mean age was on the higher end of the age range that typically experiences the highest proportion of non-contact ACL injuries, and this younger demographic may display distinct motor learning characteristics. Additionally, the sample was homogenous in terms of athletic background and current physical fitness habits. Including current high-school or collegiate student-athletes, sedentary subjects, and younger subjects would enhance the generalizability of these findings to the larger population of adolescents and young adults at risk for ACL injury. This study was designed under the assumption that TTS and DPSI are used to stratify non-contact ACL injury risk in subjects as done by DuPrey et al. (2016), but TTS and DPSI could be used to monitor ACL re-injury risk after a subject sustains an ACL injury and undergoes ACLR and these subjects may also display unique motor learning characteristics. DPS has been quantified using several other methodologies than what is presented, and it is possible that other methodologies may display different motor learning characteristics (Fransz et al., 2016; Fransz et al., 2015). The distance the subject started the BSLJL form the hurdle was not controlled for between subjects or within a subject between trials, which could affect the measurement of TTS and DPSI from trial-to-trial. Subjects were encouraged to refrain from strenuous activity 24 hours before study sessions; however, their activity level was not quantified or monitored, and it is possible that residual fatigue may have been present. Finally, the present study only investigated motor learning from the within-session perspective. Motor learning retention represents the degree to which a subject can perform a previously practiced motor control task after an elapsed amount of time. Reassessing TTS and DPSI after a short rest period, later in the same day, or on a separate day may give additional insights into motor learning.

4.5. Conclusion

Poor DPS, quantified as TTS, following a BSLJL has been prospectively associated with non-contact ACL injury risk (DuPrey et al., 2016). The simple and intuitive nature of DPS makes it ideal for wide-spread implementation as an ACL injury risk screen. Properly familiarizing a subject to the task to reduce the motor learning effect will produce the most reliable and discriminative assessment. This study demonstrated that DPS, quantified as TTS or DPSI, for the dominant leg for males may require just 1 or 2 familiarization trials on both legs to obtain optimal measurement conditions, which include the lowest (best) mean performance and lowest trial-totrial-to-trial standard deviation. But the non-dominant leg for males and both legs for females may require six or more familiarization trials on both legs, and it is possible that appreciable motor learning continues beyond the 10th trial per leg, especially for females. Based on the collective findings, it is recommended that a minimum of six familiarization trials per leg are completed and that the mean of trials 7, 8, and 9 are used for ACL injury risk assessments.

5. WITHIN AND BETWEEN DAY RELIABILITY OF INDICES OF DYNAMIC POSTURAL STABILITY

5.1. Introduction

Musculoskeletal injury risk assessments need to be reliable to allow clinicians to empirically monitor injury risk and strategically intervene prior to injury incidence (Bahr, 2016; Hopkins, 2000; Weir, 2005). The anterior cruciate ligament (ACL) is the most frequently injured knee ligament with an estimated 200,000 to 550,000 ACL injuries occurring annually in the United States (Kaeding et al., 2017; Mall et al., 2014; Musahl & Karlsson, 2019). A high proportion of these injuries are sustained by adolescents and young adults between 15 and 34 years of age during routine physical activity or athletics participation (Renstrom et al., 2008). The pooled ACL injury incidence for all athletes has been estimated to be 2.8% over a period of 1 to 25 years of athletic participation with an incidence rate of 0.69 ACL injuries per 10,000 athletic exposures (Bram et al., 2021).

It has been estimated that 40% to 70% of ACL injuries are attributed to a non-contact mechanism of injury, meaning the only appreciable force acting on the athlete immediately prior to and at the time of injury was gravity (Boden et al., 2000; Kaeding et al., 2017; Mountcastle et al., 2007; Musahl & Karlsson, 2019). ACL injury often occurs when an athlete suddenly decelerates or changes direction during the eccentric or weight absorption phase of the movement within the first 0.2 s after loading is initiated (Krosshaug et al., 2007; Olsen et al., 2004; Walden et al., 2015), which is likely why double- and single-legged jump-landings are frequently employed movements in ACL injury risk assessments. Intrinsic biomechanical and neuromuscular ACL injury risk factors that may be manifested during jump-landings include knee flexor and hip extensor weakness and/or under-recruitment combined with over-recruitment of the knee

extensors (Myer et al., 2009; Wild et al., 2013), hip abduction and external rotation weakness and/or under-recruitment (Khayambashi et al., 2016; Zazulak et al., 2005), poor trunk or core strength and/or proprioception (Zazulak et al., 2007a, 2007b), and greater dynamic knee valgus with minimal knee flexion during force attenuation (Fagenbaum & Darling, 2003; Ford et al., 2005; Hewett et al., 2005). Identifying objective indicators of ACL injury risk that appropriately capture the above mentioned neuromuscular and biomechanical risk factors is paramount. Dynamic postural stability (DPS) is one such indicator and is defined as the ability of an subject's neuromuscular system to obtain stability during a shift from a dynamic movement to a stationary position over the base of support (DuPrey et al., 2016; Liu & Heise, 2013; Ross & Guskiewicz, 2003; Wikstrom et al., 2005).

DuPrey et al. (2016) assessed DPS, quantified as time to stabilization (TTS) and defined as the time between initial contact to when vertical ground reaction force (GRF_{Vert}) reached and remained with 5% of the athlete's body mass for the remainder of the trial, following backward, forward, lateral, and medial single-legged jump-landings in collegiate student-athletes. Studentathletes who subsequently sustained a non-contact ACL injury demonstrated a baseline TTS that was 50% longer following the backwards single-legged jump-landing (BSLJL) compared to student-athletes who did not subsequently sustain an ACL injury (DuPrey et al., 2016). Baseline TTS following the forward, lateral, and medial jump-landing were not different between those that subsequently sustained a non-contact ACL injury and those that did not (DuPrey et al., 2016). It is unclear why only TTS following the BSLJL at baseline was associated with the odds of sustaining a subsequent ACL injury and TTS from the other directions did not, though it is likely that a BSLJL is a more demanding task compared to the other directions. Nevertheless, given the rarity of prospectively identified relations between an injury risk assessment and occurrence of injury, the simplicity and intuitive nature of a single measure of time, and increasing accessibility to devices that measure ground reaction force makes TTS highly appealing for widespread implementation. Therefore, further evaluation of TTS following a BSLJL is timely.

Although it has been demonstrated that different calculation methods, sampling rates, data filtering methods, and thresholds can affect TTS reliability (Fransz et al., 2016; Fransz et al., 2015), little is known about the within and between day reliability of TTS following a BSLJL specifically. It is imperative that the orthopedics and sports medicine community define TTS reliability standards because doing enhances ability SO the to make appropriate inferences about changes between repeated measurements or a subject's ACL injury risk over time. For example, following ACL reconstruction (ACLR), providers commonly assess multiple-dimensions of biomechanical and neuromuscular capacity with the underlying goal of identifying a patient's readiness to progress to the next stage of rehabilitation or return to full, unrestricted physical activity or sport participation (Burgi et al., 2019; Czuppon et al., 2014; Webster & Hewett, 2019). Similarly, providers may also want to document the efficacy of an ACL injury risk prevention program and identify those in need of additional intervention by also assessing multiple dimensions of biomechanical and neuromuscular capacity (Huang et al., 2020; Webster & Hewett, 2018). In both these use cases, characterizing the reliability of TTS during a BSLJL will allow for separation of what is "real" change in TTS, as opposed to error or noise, enhancing the discriminatory capacity of TTS as an indicator of ACL injury risk.

The primary purpose of this study is to evaluate the reliability of DPS, as measured by TTS and DPSI, following a BSLJL and assess the effect of increasing the number of BSLJL trials on reliability and the minimal detectable change (MDC). Based on previous DPS investigations (Byrne et al., 2021; Colby et al., 1999; Ebben et al., 2010; Flanagan et al., 2008; Fransz et al.,

2016; Fransz et al., 2015; Wikstrom et al., 2005), it was hypothesized that three trials per leg and the resulting mean value would elicit "moderate" to "good" reliability, as defined by a reliability coefficient between 0.50 and 0.90 (Koo & Li, 2016).

5.2. Methods

5.2.1. Research Design

This study used a crossover, semi-randomized design. Subjects completed four visits to the laboratory. Visit one, referred to as the familiarization session, always consisted of the same procedures for all subjects, which included ten familiarization trials per leg of the BSLJL. Visits two, three, and four consisted of one control session (CONTROL) and two fatigue sessions (FATIGUE₁ and FATIGUE₂) completed in a random order.

5.2.2. Subjects

Ten and fourteen recreationally active males and females, respectively, $(24.3 \pm 2.8 \text{ y}, 1.74 \pm 0.08 \text{ m}, 76.5 \pm 14.2 \text{ kg})$ between 12 and 30 years of age completed this study. This demographic was chosen as it represents subjects who experience ACL injuries. Subjects with a prior ligamentous, bony, or other soft tissue operative procedures involving the lower extremity, an orthopedic issue exacerbated by exercise, acute fracture, tumor, or infection, unfavorable cardiovascular responses to exercise, a neurological condition that effects the activation of skeletal muscle or balance, and diabetes will be excluded from the study. Additionally, active smokers or those that have smoked in the past 6 months, pregnant females, and cognitively impaired adults will also be excluded from this study. The Sanford Health Institutional Review Board approved all aspects of this study (approval number: 1009). Subjects were informed of the studies protocol, benefits, and risks before providing their informed, written, voluntary consent. None of the subjects were less than 18 years of age.

5.2.3. Procedures

5.2.3.1. Visit 1 – Familiarization Session

The subject's dominant leg was recorded as the leg in which the subject indicated they would kick a soccer ball with. The subject then completed a guided 10-minute warm up consisting of light aerobic exercise, dynamic stretching, and plyometrics. The subject then performed ten familiarization trials per leg, or twenty total trials, of the BSLJL by alternating between the dominant and non-dominant leg to avoid an acute fatigue effect with 15 seconds between trials, or 30 seconds of rest between trials performed on the same leg.

5.2.3.2. Visits 2, 3, and 4 – Control and Fatigue Sessions

Visits two, three, and four consisted of CONTROL and FATIGUE₁ and FATIGUE₂. The order of these sessions was randomized to control for an order effect. At the beginning of each of these sessions, the subject completed the same guided 10-minute warm up consisting of light aerobic exercise, dynamic stretching, and plyometrics identical to that used during visit one. The subject then completed three re-familiarization trials on each leg, or six total familiarization trials, of the BSLJL while barefoot. Following the completion of the re-familiarization trials, the subject stepped onto the force plate and remained as motionless as possible. Ground reaction force data was sampled for 10 seconds to obtain the subject's body weight (BW; N). Following a 1-minute break, the subject then performed three trials per leg, or six total trials, of the BSLJL by alternating between the dominant and non-dominant leg to avoid an acute fatigue effect with 15 seconds between trials, or 30 seconds of rest between trials performed on the same leg (PRE). During CONTROL, the subject then sat in a chair and rested for 5 minutes before re-performing three trials per leg of the BSLJL (POST) using procedures identical to PRE. After PRE during FATIGUE, the subject performed a 5-to-7-minute fatigue protocol and then re-performed three

trials per leg of the BSLJL (POST) using procedures identical to PRE; however, POST data during FATIGUE was used to address another research purpose and is not included in the present analyses.

5.2.4. Backwards Single-Legged Jump-Landing Task

The BSLJL used in this study is identical to the protocol used and described by DuPrey et al. (2016), which was adopted from Liu and Heise (2013). A 0.05 m tall hurdle was placed parallel with the edge of the force plate to normalize the minimal foot clearance off the ground required to complete the task. The subject started the task by standing on two feet directly next to a force plate with their back facing the force plate and their hands on their hips. An investigator gave the subject an audible "three, two, one, go" command. The subject then lifted the non-test leg off the ground and jumped off the test leg backwards over the hurdle and onto the force plate. The subject was instructed to land on the force plate on the test leg with their eyes focused forward and their hands on their hips, stabilize as quickly as possible, and remain motionless until the investigator indicated the trial is over. Trials were performed barefoot to minimize the stability provide by a shoe. Following the initial contact, the subject was permitted to hop or shuffle on their test leg to stabilize if their test leg foot did not contact any surrounding surface besides the force plate. Trials where the subject removed their hands from their hips upon landing, touched their non-test leg or any other body part to the ground, or contacted their test leg to any surrounding surface besides the force plate were considered failed trials. Failed trials were noted, but not repeated.

5.2.5. Data Processing

Data was processed using a custom written MATLAB program (R2021a, MathWorks, Natick, MA, USA). Raw GRF_{Vert} and horizontal ground reaction forces (GRF_{AP} and GRF_{ML}) were filtered post-hoc using a second order 12 Hz low-pass Butterworth filter (Ross et al., 2005; Webster

& Gribble, 2010). All further use of GRF data utilizes filtered GRF_{Vert}, GRF_{AP}, and GRF_{ML}. It should be noted that various digital filters have been used to process GRF data and it is recognized that different order and frequency low pass filters will elicit different DPS metrics (Fransz et al., 2015). The subject's body mass (N·9.81⁻¹; kg) was calculated as the mean GRF_{Vert} from the ten second trial performed at the beginning of the visit.

Initial contact was defined as the instant GRFvert first exceeded 20 N. GRF data was cropped into 3- and 5-second post-initial contact time frames (GRF_{Vert3}, GRF_{AP3}, and GRF_{ML3} and GRF_{Vert5}, GRF_{AP5}, and GRF_{ML5}, respectively). Time to stabilization (TTS) was calculated using GRF_{Vert5} as the length of time in seconds (Δ time; s) required for GRF_{Vert5} to reach and then remain between 95% and 105% of the subject's body weight (GRF_{Vert5}·body weight in N⁻¹; %) for the remainder of the trial (DuPrey et al., 2016). Dynamic postural stability indices were calculated using the methodology described by Wikstrom et al. (2005) and modified by (Dallinga et al., 2016; Wikstrom et al., 2010) using GRF_{Vert3}, GRF_{ML3}, and GRF_{AP3}. Stability indices for GRF_{ML} (MLSI), GRF_{AP} (APSI), and GRF_{Vert} (VSI) reflect the average magnitude of fluctuation (standard deviation) of GRF_{Vert3}, GRF_{ML3}, and GRF_{AP3} vectors around 0 N for MLSI and APSI and the subject's body weight in N for VSI. DPSI represents a composite score of MLSI, APSI, and VSI. These indices were calculated using the following equations (Dallinga et al., 2016; Wikstrom et al., 2010) where body weight is the subject's body weight in N and samples is the number of GRF data points included (e.g., 3,000 for a 3 second post-initial contact time frame if recorded at 1 kHz):

$$Medial - Lateral Stability Index = \sqrt{\sum ((0 - GRF_{ML}) \cdot Body Weight^{-1})^{2} \cdot Samples^{-1}}$$

$$Anterior - Posterior Stability Index = \sqrt{\sum ((0 - GRF_{AP}) \cdot Body Weight^{-1})^{2} \cdot Samples^{-1}}$$

$$Vertical Stability Index = \sqrt{\sum ((GRF_{Vert} - Body Weight) \cdot Body Weight^{-1})^{2} \cdot Samples^{-1}}$$

 $Dynamic \ Postural \ Stability \ Index = \sqrt{((\sum (0 - Fx)^2 + \sum (0 - Fy)^2 + \sum (Body \ Weight - Fz)^2) \cdot Body \ Weight^{-1})) \cdot Samples^{-1}}$

Data for all calculated variables were entered into a data matrix. Because "failed" trials were not repeated, cells corresponding to them were left blank. Cells corresponding to TTS were also left blank for trials in which the stability threshold, defined as reaching and remaining between 95% and 105% of the subject's body weight for the remainder of the trial, was not achieved within the 5-second post-initial contact period.

5.2.6. Statistical Analyses

The independent categorical variables of this study were leg (e.g., dominant or nondominant), session (e.g., CONTROL, FATIGUE₁, or FATIGUE₂), time (e.g., PRE or POST) and trial (e.g., 1, 2, or 3). The dependent continuous variables were TTS and DPSI.

Statistics and subsequent tables and figures were completed using R v. 4.0.5 (R Core Team, 2019) and accompanying package "gtheory" v. 0.1.2 (Moore, 2016). Descriptive statistics including means, 95% confidence intervals, medians, quartiles, minimum (25th Percentile – ($1.5 \cdot Interquartile Range$)), and maximum (75th Percentile + ($1.5 \cdot Interquartile Range$)) as well as normal Q-Q plots and histograms were used to initially explore the data.

Generalizability theory was used to assess the reliability of the dependent variables (Brennan, 1992; Shavelson & Webb, 1991). Classical test theory assumes that an observed value can be decomposed into the subject's true value and an error term. Generalizability theory extends classical test theory by recognizing that the error term can be further decomposed into one or more sources of error. In the present study, the sources of error, which are called facets in generalizability theory, include the independent categorical variables session, time, leg, and trial as well as the object of measure, the subject. In a generalizability study, the random effect variance
$(\hat{\sigma}^2)$ for each facet is estimated and partitions the error into sources. The highest order interaction facet is the residual error or left over $\hat{\sigma}^2$ that cannot be allocated to a specific source. For ideal reliability, the majority of $\hat{\sigma}^2$ should be allocated into the facet for the subject, indicating that the largest source of variation in the dependent variable is due to inherent differences between the subjects (e.g., between subject variance). Poor reliability exists if the majority of $\hat{\sigma}^2$ is allocated to other sources, especially the residual error term. The $\hat{\sigma}^2$ for the facets obtained from the generalizability is then used to conduct a decision study. A strength of a decision study is that an investigator can assess how reliability would be affected if distinct aspects of the measures were changed, such as increasing or decreasing the number of trials used. Decision studies also provide a number of relative or "norm-referenced" reliability and absolute or "domain-referenced" reliability metrics.

A four facet, fully crossed design (session: time: leg: trial) was used to perform a generalizability study and estimate $\hat{\sigma}^2$ for thirty-one facets. Relative error variance (REV; $\sum \hat{\sigma}^2$ for all facets that interact with subject except the main facet for subject) and absolute error standard deviation (AEV; $\sum \hat{\sigma}^2$ for all facets except the main facet for subject) were computed along with the relative minimal detectable change (MDC_{Rel}; $\sqrt{REV} \cdot 1.96 \cdot \sqrt{2}$), absolute minimal detectable change (MDC_{Ab}; $\sqrt{AEV} \cdot 1.96 \cdot \sqrt{2}$), generalizability coefficient ($\hat{\sigma}^2$ for the main facet for subject · ($\hat{\sigma}^2$ for the main facet for subject facet + REV^1), and dependability coefficient ($\hat{\sigma}^2$ for the main facet for subject · ($\hat{\sigma}^2$ for the main facet for subject facet + AEV)⁻¹). The generalizability and dependability coefficients are mostly analogous to the interclass correlation coefficient (ICC), with greater coefficients representing greater reliability. Although there are no guidelines describing coefficient thresholds (e.g., "poor", "moderate", etc.) for the generalizability and dependability coefficients is specifically and even deducing ICC values into these qualitative ranges is very context

specific, to aid in interpreting the coefficients presented here a value less than 0.50 is considered poor, 0.50 to 0.75 is considered moderate, 0.75 to 0.90 is considered good, and greater than 0.90 is considered excellent (Koo & Li, 2016). A specific aim of this paper is to determine the reliability of the dependent variables when different numbers of trials per leg are performed. Based on classical test theory and the assumption of random error, as additional trials are performed and averaged together, the subject's mean value form the measured trials and their true but unknown value get closer together and the error term decreases resulting in improved reliability. Decision studies were therefore completed by adjusting the number of days, sessions, and times for testing to one and varying the number of trials performed per leg (e.g., 1, 2, 3...10) and re-computing the reliability metrics. No probability or hypothesis testing is conducted with a generalizability or decision study.

5.3. Results

Normal Q-Q and histograms plots for TTS are presented in Figure 12. TTS was not normally distributed and appears to be positively skewed. A boxplot and means with 95% confidence intervals for TTS over each level of the independent variables are presented in Figure 13. The TTS global mean and standard deviation was 1.287 ± 0.874 s, the range was 0.270 - 4.992 s, and the 25^{th} , 50^{th} (median), and 75^{th} quartiles were 0.705, 1.028, and 1.498 s, respectively. The estimated random standard deviation components are presented in Figure 14. The residual had the largest estimated random standard deviation (62.5%), which is composed of error from the independent variables that cannot be isolated or unidentified sources of error that were not captured by the independent variables. Subject: session: leg: time (17.8%), subject (13.3%), subject: session (5.7%), and session: trial (0.6%) were the only other facets with estimated standard deviation. Using the conditions testing occurred in (e.g., leg = 2, session = 3, time =2, and trial =3), the

 MDC_{Rel} and MDC_{Ab} are 0.559 and 0.603 s, respectively, and the generalizability and dependability coefficients are 0.686 and 0.683, respectively. The relative and absolute reliability metrics for TTS are presented in Figure 15 for over varying numbers of trials per leg within a single testing session at a single time. Absolute and relative reliability metrics were very similar because a small proportion of standard deviation (0.6%) was attributed to a facet that did not contain an interaction with the subject facet (e.g., session: trial).

Figure 12

Normal quantile-quantile plot and histogram for time to stabilization (TTS). The vertical dashed line in the histogram represents the global mean for TTS



Boxplot and mean and 95% confidence interval for time to stabilization (TTS)



Generalizability study random effect standard deviation for facets for time to stabilization (TTS)



Generalizability Study Random Effect Variance





Normal Q-Q and histograms plots for DPSI are presented in Figure 16. DPSI was also not normally distributed and appears to be positively skewed. A boxplot and means with 95% confidence intervals for DPSI over each level of the independent variables are presented in Figure 17. The DPSI global mean and standard deviation was 2.057 ± 0.426 , the range was 1.208 - 3.887, and the quartiles were 1.753, 1.997, and 2.225, respectively. The estimated random standard deviation components are presented in Figure 18. The subject facet had the largest estimate random standard deviation (61.3%), which suggests that standard deviation in DPSI is highly attribute to the subject inherent DPSI capacity. The residual had the next largest estimated random standard deviation (27.1%), which is composed of error from the independent variables that cannot be isolated or unidentified sources of error that were not captured by the independent variables. Subject: trial: leg: time (4.4%), subject: leg (2.6%), subject: session (2.1%), and session: trial: leg (0.8%), trial (0.5%), subject: trial: leg (0.4%), session (0.4%), and time (0.4%) were the only other facets with estimated standard deviation. Using the conditions testing occurred in (e.g., leg = 2, session = 3, time =2, and trial =3), the MDC_{Rel} and MDC_{Ab} are 0.229 and 0.245, respectively, and the generalizability and dependability coefficients are 0.944 and 0.936, respectively. The relative and absolute reliability metrics for DPSI are presented in Figure 19 for over varying numbers of trials per leg within a single testing session at a single time. Again, absolute and relative reliability metrics were similar because a small proportion of standard deviation (2.1%) was attributed to a facet that did not contain an interaction with the subject facet.





Boxplot and mean and 95% confidence interval for dynamic postural stability index (DPSI)

	Box	(plot
	Fatigue2 : Pre : Non-Dominant : Trial 3	
Session:Time:Leg:Trial	Fatigue2 : Pre : Non-Dominant : Trial 2 -	0-0-0 00 00 00 0-0-0 0
	Fatigue2 : Pre : Non-Dominant : Trial 1 -	**************************************
	Fatigue2 : Pre : Dominant : Trial 3 -	
	Fatigue2 : Pre : Dominant : Trial 2	******
	Fatigue2 : Pre : Dominant : Trial 1 -	**** - 0 00 0 -*0 0
	Fatigue1 : Pre : Non-Dominant : Trial 3	······································
	Fatigue1 : Pre : Non-Dominant : Trial 2 -	0
	Fatigue1 : Pre : Non-Dominant : Trial 1 -	0-000-000 0 0 -00-00 0 0
	Fatigue1 : Pre : Dominant : Trial 3 -	•
	Fatigue1 : Pre : Dominant : Trial 2 -	0-0-0-0-0-0-0-0-0-0-0-0-0-0-0-0-0-0-0-
	Fatigue1 : Pre : Dominant : Trial 1 -	0-00-00 000 0 0 0 0 0 0 0 0 0 0 0 0 0 0
	Control : Pre : Non-Dominant : Trial 3 -	······································
	Control : Pre : Non-Dominant : Trial 2	0-000-000 0 0 0-0-0-0-0
	Control : Pre : Non-Dominant : Trial 1 -	0.00000 000 0 0 0 0 0 0 0 0 0 0 0 0 0 0
	Control : Pre : Dominant : Trial 3 -	amo o a a a a a a a
	Control : Pre : Dominant : Trial 2 -	0-0-00-0 0 0 00 00-00-0 0
	Control : Pre : Dominant : Trial 1 -	0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0
	Control : Post : Non-Dominant : Trial 3	0-0-0-00 00 00 0-0-0-0-00
	Control : Post : Non-Dominant : Trial 2 -	e-m-m e m e e e e e e
	Control : Post : Non-Dominant : Trial 1	
	Control : Post : Dominant : Trial 3 -	
	Control : Post : Dominant : Trial 2 -	0
	Control : Post : Dominant : Trial 1 -	
	0	1 2 3 4
		DPSI

Mean and 95% Confidence Interval



Generalizability study random effect standard deviation for facets for dynamic postural stability index (DPSI)



Generalizability Study Random Effect Variance

Relative (grey) and absolute (black) minimal detectable change (MDC), generalizability coefficient (grey), and dependability coefficient (black) for dynamic postural stability index (DPSI)



5.4. Discussion

Characterizing the reliability of TTS and DPSI during a BSLJL will allow for separation of what is "real" change in TTS and DPSI, as opposed to error, enhancing their discriminatory capacity. Based on previous DPS investigations (Byrne et al., 2021; Colby et al., 1999; Ebben et al., 2010; Flanagan et al., 2008; Fransz et al., 2016; Fransz et al., 2015; Wikstrom et al., 2005), it was hypothesized that three trials per leg and the resulting mean value would elicit "moderate" to "good" reliability, as defined by a reliability coefficient between 0.50 and 0.90 (Koo & Li, 2016). For DPSI, this hypothesis was supported because DPSI demonstrated good reliability after 2 trials and excellent reliability after 6 trials per leg. However, for TTS, this hypothesis was not supported because even after 10 trials per leg TTS still demonstrated poor reliability.

TTS and DPSI were positively skewed, suggesting that TTS and DPSI for most subjects' trials regardless of session, time, or leg were relatively small (better). However, there were some subjects or trials within a subject that demonstrated higher (worse) TTS and DPSI. For TTS, the largest source of variance (62.5%) was attributed to the residual, which represents the highest order interaction term between subject, session, time, leg, and trial compounded with error. Essentially, 62.5% of the total variance in TTS is due to unexplained systematic or unsystematic (random) errors. The second highest source of variance (17.8%) was attributed to the interaction between subject, session, time, and leg. This suggests that when averaged over trials, the subjects displayed different TTS depending on the session, time, and leg. This finding is particularly troublesome because it is likely that in practice many clinicians would average TTS from several trials together, yet this finding indicates that this average will vary despite identical testing conditions. Finally, the subject facet has the third highest variance (13.3%) attributed to it. This suggests that 13.3% of the total variance in TTS is due to inherent differences between subjects in their DPS, which is

the ideal source of systematic variance and should be closer to 100% for adequate reliability. In comparison, for DPSI, the subject facet was the highest source of variance (61.3%) indicating that the overwhelming source of total variance was the inherent difference between subjects in their DPS and the residual (27.1%) explained nearly all the remaining total variance in DPSI. This finding alone suggests that DPSI is much more reliable and discriminative than TTS.

In practice, one is likely to assess a subject's TTS or DSPI on both legs within a single testing session and time and not over multiple sessions and times. In these instances, TTS and DPSI could be re-assessed later to monitor ACLR rehabilitation progress and return to play readiness or the effect of an intervention such as an ACL injury prevention program. In these use cases, it is critical to identify and define what "real" change is between these testing occurrences, as opposed to error, and the MDC defines this critical threshold. Further, under the assumption that a proportion of error is random, as the number of measurements is increased and these new measures are averaged together with the previous measures, the difference between this mean value and the subject's true but unknown value as well as the magnitude of the error term decreases. This suggests that additional trials per leg within a single testing session and time will decrease (improve) the MDC and identifying the number of trials required to obtain sufficient reliability will lead to the most time efficient, reliable, and therefore discriminative assessment. With both TTS and DPSI, improvements in the reliability coefficients and the MDC were observed with additional trials. However, even with 10 trials per leg, TTS reliability was poor (e.g., < 0.50for both reliability coefficients) and the MDC exceed 1.0 s indicating that a subject's TTS would need to change by over 1.0 s to be considered "real". This MDC value is quite large considering the global mean TTS for the present study was 1.287 s and for DuPrey et al. (2016) was 1.105 s. Conversely, DPSI demonstrated good reliability after 2 trials (e.g., > 0.75) and excellent reliability (e.g., > 0.90) after 6 trials per leg. At 6 trials, the MDC is approximately 0.30, which is much smaller than the global mean DPSI of 2.057 in the present study.

DPS has been quantified using several other methodologies than what was utilized here, and it is possible that other methodologies that use different trial lengths, GRF components, input signals, stability thresholds, and stability definitions may display different reliability standards (Fransz et al., 2016; Fransz et al., 2015). There are no studies that have investigated the reliability of TTS and DPSI, as calculated in the present study, following a BSLJL specifically to directly compare findings to. Previous studies reporting reliability standards for TTS, specifically calculated using GRF_{vert}, have found ICCs of 0.83 for 10 trials, 0.69 for 3 trials, 0.64 for 3 trials, and 0.42 for a single trial (Colby et al., 1999; Ebben et al., 2010; Flanagan et al., 2008). Byrne et al. (2021) assessed the within and between day reliability of TTS, also specifically calculated using GRF_{Vert}, following a forward single-legged drop jump-landing and reported the MDC. The authors demonstrated a within day ICC of 0.715 and an MDC between trials of 0.17 s and a between day ICC of 0.830 and an MDC of 0.12 s. However, the authors took the three fastest (best) trials of the four trials measured per day, and this practice artificially inflates the reliability by removing a potential "outlier." Nevertheless, the poorer reliability between trials in a given day compared to between days is insightful, as it corroborates the high degree of random error observed in the present study. Ultimately, it appears that the reliability observed in the present study is worse than the reliability found in other studies using a similar TTS computational methodologies, and it is unclear if the inferior reliability in the present study is due to different motor control tasks (e.g., BSLJL vs forward single-legged jump-landing), computational methods, or other unexplained reasons. Wikstrom et al. (2005), the first to describe the DPSI computational method, demonstrated a between day ICC of 0.96, which is comparable to the reliability demonstrated in the present

study. However, Wikstrom et al. (2005) did not correct DPSI for the subject's body weight. A subject with greater body weight would display a greater DPSI, which would cause some of the total variance in DPSI to be artificially shifted to the subject facet (e.g., between subject variance), simply because some subjects weigh more or less than others, and the resulting reliability is potentially inflated.

This study featured a separate familiarization session where the subjects performed ten trials of the BSLJL per leg. Although, there is evidence that motor learning did occur over the ten trials per leg as an evident by improved performance and reduced trial-to-trial-to-trial standard deviation, it is unclear if this sufficiently reduced the motor learning effect in that session (Chapter 4). Subjects were granted three re-familiarization trials at the beginning of each subsequent visits, which were used to compute the reliability coefficients and MDC for the present study, but these were unmeasured. Measuring these trials would have given insight into motor learning retention between visits or if the motor learning effect was sufficiently reduced by these three refamiliarization sessions. Ensuring the motor learning effect is sufficiently reduced, but likely not eliminated, is important because in a statistical sense any familiarization trials prior to measured trials of motor control task performance shifts a proportion of the variance in performance from between trials within a subject (e.g., subject:trial) to between subjects (e.g., subject), which results in improved reliability and therefore discriminative capacity. Nevertheless, other studies did not provide such a robust separate familiarization session, so it potentially indicates that the reliability described in the present study is under optimal conditions and reliability would have been worse had this session not been completed; although, this is speculation.

One unique aspect of this study was that failed trials were noted but not repeated. Other studies, including DuPrey et al. (2016), repeated failed trials, possibly to ensure that each subject

has an identical number of trials available that can be either averaged together or used in a number of statistical analyses that require complete observations (e.g., ANOVA). However, subjects who perform additional trials due to failures may display better DPS on subsequent successful trials simply because they had more familiarization trials, and by consequence this could mischaracterize a subject. Alternatively, a subject who fails multiple trials but has the same mean TTS or DPSI as a subject who successfully completes all trials would have their risk stratified similarly. Although, the underlying biomechanical or neuromuscular reasons for the failed trials may have some meaning in the context of ACL injury risk. Nevertheless, the inability to meaningfully incorporate failed trials into the statistical analyses represents a limitation that extends beyond the present study.

There are several limitations of this study and possibilities for future research. The subjects in the present study represented a homogenous group of college aged, recreationally active subjects. Including a larger sample of adolescent and young adult subjects that represent a greater proportion of the 15 and 34 year of old age group, as this is the age range that most frequently sustained ACL injury (Renstrom et al., 2008), who are competitive athletes, recreationally active, and sedentary would enhance the generalizability of these reliability findings. Further, this study excluded subjects with a history of significant unilateral pathology, such as an ACL injury and subsequent ACLR, but these subjects may display unique degrees of reliability that may change the definition of "real" change. Further, the distance the subject started the BSLJL form the hurdle was not controlled for between subjects or within a subject between trials, which could affect the measurement of TTS and DPSI. Finally, subjects were encouraged to refrain from strenuous activity 24 hours before study sessions; however, their activity level was not quantified or monitored, and it is possible that residual fatigue may have been present.

5.5. Conclusion

Poor DPS, quantified as TTS, following a BSLJL has been prospectively associated with non-contact ACL injury risk (DuPrey et al., 2016). The simple and intuitive nature of DPS makes it ideal for wide-spread implementation as an ACL injury risk screen. Characterizing the reliability of TTS and DPSI, another popular indicator of DPS, during a BSLJL will allow for separation of what is "real" change in TTS and DPSI, as opposed to error, and enhance the ability to interpret longitudinal changes in ACL injury risk. This study demonstrated that DPSI has good to excellent reliability with approximately six trials per leg required to sufficiently reduce the MDC. However, TTS demonstrated poor reliability. Even after the completion of ten trials per leg, TTS could only detect substantial changes. DPSI is therefore a more reliable indicator of DPS, although it has not been prospectively associated with ACL injury, whereas TTS has.

6. EFFECT OF FATIGUE AND HIP EXTERNAL ROTATION AND ABDUCTION STRENGTH ON DYNAMIC POSTURAL STABILITY

6.1. Introduction

Characterizing the relations between ACL injury risk factors, which may include biological sex, poor dynamic postural stability (DPS), inadequate proximal hip strength, and fatigue may allow providers to design high fidelity ACL injury risk screening tools, empirically monitor ACL injury risk, and strategically intervene prior to ACL injury incidence. The knee accounts for nearly 60% of all sport-related surgeries in high school aged athletes (Ingram et al., 2008) and the anterior cruciate ligament (ACL) is the most frequently injured knee ligament (Kaeding et al., 2017; Mall et al., 2014; Musahl & Karlsson, 2019). The incidence of ACL injury is highest for adolescents and young adults and peaks at 16 to 18 years of age, with a high proportion of ACL injuries occurring during athletic or physical activity participation resulting from a non-contact mechanism of injury (Musahl & Karlsson, 2019). There are a multitude of risk factors for ACL injury that form a complex network, which makes stratifying ACL injury risk extremely challenging (Alentorn-Geli et al., 2014; Ardern et al., 2018; Bayer et al., 2020; Bourne et al., 2019; Hewett et al., 2006).

Biological sex is a strong risk factor for ACL injury as female athletes have a two- to fourfold greater relative risk for ACL injury compared to their similarly trained male counterparts (Agel et al., 2016; Huston et al., 2000). This disparity in risk between female and male athletes is likely driven by several risk factors (Huston et al., 2000). Females tend to demonstrate reduced relative muscle strength and more aberrant biomechanics associated with ACL injury compared to males, although the presence of the same risk factors in males also increases their risk for ACL injury (Boden et al., 2010; Hewett et al., 2006; Krosshaug et al., 2007).

DPS is defined as the ability of a subject's neuromuscular system to obtain stability during a shift from a dynamic movement to a stationary position over the base of support (DuPrey et al., 2016; Liu & Heise, 2013; Ross & Guskiewicz, 2003; Wikstrom et al., 2005). DuPrey et al. (2016) assessed DPS, quantified using ground reaction force (GRF) data as time to stabilization (TTS) defined as the time between initial contact to when vertical GRF (GRF_{Vert}) reached and remained with 5% of body mass for the remainder of the trial, following backward, forward, lateral, and medial single-legged jump-landings in collegiate student-athletes. Student-athletes who subsequently sustained a non-contact ACL injury demonstrated a baseline TTS that was 50% longer following the backwards single-legged jump-landing (BSLJL) compared to student-athletes who did not subsequently sustain an ACL injury (DuPrey et al., 2016). Baseline TTS following the forward, lateral, and medial jump-landing were not different between those that subsequently sustained a non-contact ACL injury and those that did not (DuPrey et al., 2016). It is unclear why only TTS following the BSLJL at baseline was associated with the odds of sustaining a subsequent ACL injury and TTS from the other directions did not, though it is likely that a BSLJL is a more demanding task compared to the other directions (Hron et al., 2020). DPS following a singlelegged jump-landing has been used to assess post-ACL reconstruction (ACLR) and ACL deficient subjects and demonstrated sufficient sensitivity to detect involved limb deficits in neuromuscular control (Colby et al., 1999; Webster & Gribble, 2010). Several studies have also utilized DPS following a single-legged jump-landing to compare neuromuscular control between males and females with contrasting findings, although these studies used heterogeneous motor control tasks and DPS computational methodologies (Dallinga et al., 2016; Ebben et al., 2010; Lephart et al., 2002; Wikstrom et al., 2006).

Adequate proximal hip strength is essential for counteracting distal neuromuscular and biomechanical aberrancies, such as dynamic knee valgus which is in part caused by hip adduction and internal rotation (Powers, 2010). Khayambashi et al. (2016) assessed the isometric hip external rotation and abduction strength of 501 male and female athletes using a hand-held dynamometer and then prospectively monitored the athletes for one year for ACL injury. Fifteen non-contact ACL injuries occurred during the monitoring period and analyses revealed that poor hip strength normalized to body weight was associated with subsequent ACL injury risk. It is well established that female adolescents and young adults have lower strength to body weight ratios compared to males (Beutler et al., 2009), which is one potential reason for the higher ACL injury risk observed in female athletes. Further, although the relation between strength and DPS is unclear (Lephart et al., 2002; Williams et al., 2016), several studies have demonstrated that individuals with poor strength display aberrant biomechanics during a single-legged jump-landing that are associated with ACL injury risk (Bandholm et al., 2011; Neamatallah et al., 2020; Suzuki et al., 2015; Zazulak et al., 2005).

Finally, fatigue, although challenging to define (Enoka & Duchateau, 2008; Enoka & Stuart, 1992), transiently reduces a subject's neuromuscular capacity to control, attenuate, and transfer force during motor control tasks (Johnston III et al., 1998; Paillard, 2012). Despite this, research has not identified a clear association between fatigue and an actual increased risk for ACL injury occurrence (Barber-Westin & Noyes, 2017; Bourne et al., 2019; Doyle et al., 2019). This may be because the ecological validity of fatigue manifested in well controlled laboratory studies is poor and studies tend to report a heterogeneous sample of risk factors as primary outcome variables (Barber-Westin & Noyes, 2017; Bourne et al., 2019; Doyle et al., 2019). Nevertheless, there is strong evidence that fatigue, no matter how it is induced, results in decrements in DPS

following a single-legged jump-landing (Bond et al., 2020; Shaw et al., 2008; Wikstrom et al., 2004). Further, it is well documented that fatigue increases the presence of aberrant neuromuscular and biomechanical risk factors associated with ACL injury (Benjaminse et al., 2008; Borotikar et al., 2008; Chappell et al., 2005; Chavez et al., 2013; Cortes et al., 2014; Kernozek et al., 2008; McLean et al., 2007; Mclean & Samorezov, 2009; Santamaria & Webster, 2010).

The primary purpose of this study was to evaluate the relation between biological sex, fatigue, hip external rotation and abduction strength, and DPS as measured by TTS and DPSI. Based on previous investigations it was hypothesized that DPS would be compromised after completion of a short, high-intensity exercise bout (Bond et al., 2020; Shaw et al., 2008; Wikstrom et al., 2004) and that subjects with greater hip strength will demonstrate superior DPS (Bandholm et al., 2011; Lephart et al., 2002; Neamatallah et al., 2020; Suzuki et al., 2015; Williams et al., 2016; Zazulak et al., 2005).

6.2. Methods

6.2.1. Research Design

This study used a crossover, semi-randomized design. Subjects completed four visits to the laboratory. Visit one, referred to as the familiarization session, always consisted of the same procedures for all subjects, which included ten familiarization trials per leg of the BSLJL. Visits two, three, and four consisted of one control session (CONTROL) and two fatigue sessions (FATIGUE₁ and FATIGUE₂) completed in a random order.

6.2.2. Subjects

Ten and fourteen recreationally active males and females, respectively, $(24.3 \pm 2.8 \text{ y}, 1.74 \pm 0.08 \text{ m}, 76.5 \pm 14.2 \text{ kg})$ between 12 and 30 years of age completed this study. This demographic was chosen as it represents subjects who experience ACL injuries. Subjects with a prior

ligamentous, bony, or other soft tissue operative procedures involving the lower extremity, an orthopedic issue exacerbated by exercise, acute fracture, tumor, or infection, unfavorable cardiovascular responses to exercise, a neurological condition that effects the activation of skeletal muscle or balance, and diabetes will be excluded from the study. Additionally, active smokers or those that have smoked in the past 6 months, pregnant females, and cognitively impaired adults will also be excluded from this study. The Sanford Health Institutional Review Board approved all aspects of this study (approval number: 1009). Subjects were informed of the studies protocol, benefits, and risks before providing their informed, written, voluntary consent. None of the subjects were less than 18 years of age.

6.2.3. Procedures

6.2.3.1. Visit 1 – Familiarization Session

The subject had their anthropometrics including height, body mass, right leg length, and right shank length measured. Right leg length was measured from the anterior superior iliac spine to medial malleolus and shank length was measured from the lateral knee joint line to lateral malleoli measured using a tape measure while the subject was in the supine position (Myer et al., 2010). Right thigh length was then calculated as the difference between right leg length and right shank length. Leg and shank length were only measured on the right side as they are not expected to be appreciably different to the left side in this healthy population. Leg dominance was also recorded as the leg the subject indicated they would kick a soccer ball with. The subject then completed a guided 10-minute warm up consisting of light aerobic exercise, dynamic stretching, and plyometrics (Table 1). The subject then had their bilateral hip external rotation and abduction strength assessed using procedures described in detail below. The subject then performed ten familiarization trials per leg, or twenty total trials, of the BSLJL by alternating between the

dominant and non-dominant leg to avoid an acute fatigue effect with 15 seconds between trials, or 30 seconds of rest between trials performed on the same leg. To conclude visit 1, the subject had their maximal vertical reach and jump measured while shod to standardize the jump height used for the countermovement jumps in the fatigue protocol used during the fatigue session, which is explained in detail below (Chappell et al., 2005; Cortes et al., 2012). Following measurement of vertical reach, each subject was given three vertical jump attempts with 30 seconds of rest between attempts. The subject's maximal vertical jump was calculated as the difference in height between their vertical reach and their best jump height.

6.2.3.2. Visits 2, 3, and 4 – Control and Fatigue Sessions

Visits two, three, and four consisted CONTROL, FATIGUE₁ and FATIGUE₂. The order of these sessions was randomized to control for an order effect. At the beginning of each of these sessions, the subject completed the same guided 10-minute warm up consisting of light aerobic exercise, dynamic stretching, and plyometrics identical to that used during visit one familiarization session. The subject then completed three re-familiarization trials on each leg, or six total trials, of the BSLJL while barefoot. Following the completion of the re-familiarization trials, the subject stepped onto the force plate and remained as motionless as possible. Ground reaction force data was sampled for 10 seconds to obtain the subject's body weight (BW; N). Following a 1-minute break, the subject then performed three trials per leg, or six total trials, of the BSLJL by alternating between the dominant and non-dominant leg to avoid an acute fatigue effect with 15 seconds between trials, or 30 seconds of rest between trials performed on the same leg (PRE). During the control sessions, the subject then sat in a chair and rested for 5 minutes before re-performing three trials per leg of the BSLJL using procedures identical to PRE (POST). During the fatigue sessions, the subject then performed a 5-to-7-minute fatigue protocol before re-performing three trials per

leg of the BSLJL using procedures identical to PRE (POST); however, POST data during the fatigue session was used to address another research purpose and is not included in the present analyses.

6.2.4. Backwards Single-Legged Jump-Landing Task

The BSLJL used in this study is identical to the protocol used and described by DuPrey et al. (2016), which was adopted from Liu and Heise (2013). A 0.05 m tall hurdle was placed parallel with the edge of the force plate to normalize the minimal foot clearance off the ground required to complete the task. The subject started the task by standing on two feet directly next to a force plate with their back facing the force plate and their hands on their hips. An investigator gave the subject an audible "three, two, one, go" command. The subject then lifted the non-test leg off the ground and jumped off the test leg backwards over the hurdle and onto the force plate. The subject was instructed to land on the force plate on the test leg with their eyes focused forward and their hands on their hips, stabilize as quickly as possible, and remain motionless until the investigator indicated the trial is over. Trials were performed barefoot to minimize the stability provide by a shoe. Following the initial contact, the subject was permitted to hop or shuffle on their test leg to stabilize if their test leg foot did not contact any surrounding surface besides the force plate. Trials where the subject removed their hands from their hips upon landing, touched their non-test leg or any other body part to the ground, or contacted their test leg to any surrounding surface besides the force plate were considered failed trials. Failed trials were noted, but not repeated.

6.2.5. Hip External Rotation and Abduction Strength Assessment

The subject was assessed for bilateral hip external rotation and abduction strength using the procedures described by Khayambashi et al. (2016) using a hand-held manual muscle tester (Model 01165, Lafayette Instrument, Lafayette, IN, USA) sampling at 40 Hz. To assess external rotation strength, the subject sat on the edge of an exam table with their knees flexed to 90° and the hand-held manual muscle tester was placed just proximal to the medial malleolus of the test leg. To assess abduction strength, the subject laid on their side with their back supported by a firm surface and their hip abduct to approximately 30° and the hand-held manual muscle tester was placed just proximal to the lateral femoral epicondyle of the test leg. For both external rotation and abduction strength assessments, the investigator provided a "three, two, one, go" command and then the subject performed a maximal voluntary isometric contraction. The muscle tester began recording when the subject's contraction force first exceeded 22 N and data was then sampled for 3 seconds after this instance. Three trials were performed per leg per assessment with a minimum of 15 seconds of rest between trials. Peak force (N) following each trial was recorded and averaged for the three trials per leg per assessment and used for subsequent calculations. Relative peak force was calculated as the ratio of peak force (N) to body weight (BW; N) (N·BW⁻¹) and illustrated as a percentage as reported by Khayambashi et al. (2016). Relative peak torque was calculated as the product of peak force (N) and the moment arm (m), which for hip external rotation and abduction are estimated as the right shank length and right thigh length, respectively, and divided by body mass (kg) $(N \cdot m \cdot kg^{-1})$, which represents an additional degree of allometric scaling (Bazett-Jones et al., 2011). Relative peak force and torque were then averaged between the dominant and nondominant leg for abduction and external rotation separately and used for analysis.

6.2.6. Fatigue Protocol

The fatigue protocol the subjects completed was identical to the procedure described by Cortes et al. (2012) in the shod condition. This functional fatigue consisted of a series of exercises, which included step-ups on a 0.3-m tall box, an "L-drill," 5 countermovement vertical jumps reaching to 80% of the subject's maximal vertical jump height that was identified during visit one, and agility drills on an agility ladder. The fatigue protocol started by having the subject perform a series of step-up movements onto a 0.3-m box for 20-seconds (McLean et al., 2007). Immediately after, the subject performed one repetition of the L-drill. This drill was performed by placing three cones on the ground in an "L" formation 4.5-m apart. The subject then sprinted around the cones in a standardized order making an "L" shape, which has been described previously (Sierer et al., 2008). Following the L-drill, the subject completed five countermovement vertical jumps reaching up to touch a marker set at 80% of their maximal vertical jump height identified during visit one. Finally, the subject completed agility drills on an agility ladder, which consisted of sprinting forwards, backwards, or laterally and placing each foot in a designated section of the 10-yard ladder. This entire sequence of four exercises will be completed four times, with no rest between rounds. Heart rate will be monitored through the fatigue protocol using the Polar heart rate monitor. The subject will be considered fatigued if their heart rate is greater than 85% of their estimated maximal heart rate, considered as 220 beats per minute minus their age, at any point during the fatigue protocol. The functional fatigue takes approximately 5 to 7 minutes to complete. At the conclusion of the fatigue protocol, the subject will quickly take of their shoes and return to the force plate. The first POST trial will be initiated within 30 seconds of completing the fatigue protocol.

6.2.7. Data Processing

Data was processed using a custom written MATLAB program (R2021a, MathWorks, Natick, MA, USA). Raw GRF_{Vert} and horizontal ground reaction forces (GRF_{AP} and GRF_{ML}) were filtered post-hoc using a second order 12 Hz low-pass Butterworth filter (Ross et al., 2005; Webster & Gribble, 2010). All further use of GRF data utilizes filtered GRF_{Vert}, GRF_{AP}, and GRF_{ML}. It should be noted that various digital filters have been used to process GRF data and it is recognized

that different order and frequency low pass filters will elicit different DPS metrics (Fransz et al., 2015). The subject's body mass (N \cdot 9.81⁻¹; kg) was calculated as the mean GRF_{Vert} from the ten second trial performed at the beginning of the visit.

Initial contact was defined as the instant GRFvert first exceeded 20 N. GRF data was cropped into 3- and 5-second post-initial contact time frames (GRF_{Vert3}, GRF_{AP3}, and GRF_{ML3} and GRF_{Vert5}, GRF_{AP5}, and GRF_{ML5}, respectively). Time to stabilization (TTS) was calculated using GRF_{Vert5} as the length of time in seconds (Δ time; s) required for GRF_{Vert5} to reach and then remain between 95% and 105% of the subject's body weight (GRF_{Vert5}·body weight in N⁻¹; %) for the remainder of the trial (DuPrey et al., 2016). Dynamic postural stability indices were calculated using the methodology described by Wikstrom et al. (2005) and modified by (Dallinga et al., 2016; Wikstrom et al., 2010) using GRF_{Vert3}, GRF_{ML3}, and GRF_{AP3}. Stability indices for GRF_{ML} (MLSI), GRFAP (APSI), and GRFvert (VSI) reflect the average magnitude of fluctuation (standard deviation) of GRF_{Vert3}, GRF_{ML3}, and GRF_{AP3} vectors around 0 N for MLSI and APSI and the subject's body weight in N for VSI. DPSI represents a composite score of MLSI, APSI, and VSI. These indices were calculated using the following equations (Dallinga et al., 2016; Wikstrom et al., 2010) where body weight is the subject's body weight in N and samples is the number of GRF data points included (e.g., 3,000 for a 3 second post-initial contact time frame if recorded at 1 kHz):

$$Medial - Lateral Stability Index = \sqrt{\sum ((0 - GRF_{ML}) \cdot Body Weight^{-1})^{2} \cdot Samples^{-1}}$$

$$Anterior - Posterior Stability Index = \sqrt{\sum ((0 - GRF_{AP}) \cdot Body Weight^{-1})^{2} \cdot Samples^{-1}}$$

$$Vertical Stability Index = \sqrt{\sum ((GRF_{Vert} - Body Weight) \cdot Body Weight^{-1})^{2} \cdot Samples^{-1}}$$

$$Dynamic Postural Stability Index = \sqrt{((\sum (0 - Fx)^{2} + \sum (0 - Fy)^{2} + \sum (Body Weight - Fz)^{2}) \cdot Body Weight^{-1})) \cdot Samples^{-1}}$$

Data for all calculated variables were entered into a data matrix. Because "failed" trials were not repeated, cells corresponding to them were left blank. Cells corresponding to TTS were also left blank for trials in which the stability threshold, defined as reaching and remaining between 95% and 105% of the subject's body weight for the remainder of the trial, was not achieved within the 5-second post-initial contact period.

6.2.8. Statistical Analyses

The independent categorical variables of this study were biological sex (e.g., male or female) leg (e.g., dominant or non-dominant), session type (e.g., CONTROL or FATIGUE), and time (e.g., PRE or POST). The independent continuous variables of this study were relative peak force and torque for hip abduction and external rotation. The dependent continuous variables were TTS and DPSI. Statistics and subsequent tables and figures were completed using R v. 4.0.5 (R Core Team, 2019) and accompanying packages "lme4" v. 0.1.2 (Bates et al., 2022) and "emmeans" v. 1.7.2 (Lenth et al., 2022). Descriptive statistics including means, 95% confidence intervals, medians, quartiles, minimum (25th Percentile – ($1.5 \cdot$ Interquartile Range)), and maximum (75th Percentile + ($1.5 \cdot$ Interquartile Range)) as well as normal Q-Q plots and histograms were used to initially explore the data.

TTS and DPSI were assessed using separate linear mixed-effects models where a full factorial of session type (e.g., CONTROL and FATIGUE), time (e.g., PRE and POST), and biological sex (e.g., male and female) were entered as fixed-effects and each subject was assigned a random intercept. If appropriate, Bonferroni corrected post-hoc tests were performed to identify the source of the effect for any significant effects.

TTS and DPSI were then aggregate by subject for CONTROL, PRE FATIGUE, and POST FATIGUE separately. The difference (PRE-POST) between each subject's aggregated TTS and

DPSI from PRE to POST FATIGUE was also computed by subtracting their POST mean from PRE mean. Pearson correlations were then used to assess the relation between hip abduction and external rotation force and torque and TTS and DPSI during CONTROL, POST FATIGUE, and PRE-POST FATIGUE. Significance was set to p < 0.05 for all statistical analyses.

6.3. Results

Hip abduction and external rotation force and torque are presented in Figure 20.

Means and 95% confidence intervals for relative peak force and relative peak torque for hip abduction and external rotation for males (blue) and females (pink) on the dominant and nondominant leg. Whiskers: 95% confidence interval; large dot: mean; small dots: subjects



There was a total of 814 trials available for analysis. Fifty trials (5.8%) were considered failed. The failed trials were evenly distributed between CONTROL (n = 16), FATIGUE1 (n = 19), and FATIGUE2 (n = 15) as well as between PRE (n = 21) and POST (n = 29). Histogram and normal Q-Q plots for TTS are presented in Figure 21. TTS is not normally distributed and appears positively skewed, indicating that most trials are relatively low (better) with less frequent higher (worse) trials. Boxplots presenting the median, quartiles, minimum, and maximum and means with 95% confidence intervals are presented in Figure 22.

Histogram and normal Q-Q plot for males (blue) and females (pink) for time to stabilization on the dominant and non-dominant leg



Boxplot and mean and 95% confidence interval for time to stabilization for males (blue) and females (pink) before (PRE) and after (POST) a control session and two fatigue sessions on the dominant and non-dominant leg



The linear mixed-effects model evaluating TTS revealed an effect of session type (F = 6.073, p = 0.013) as TTS was higher (worse) during FATIGUE than CONTROL (Figure 23). The model also revealed an effect of biological sex × session (F = 4.106, p = 0.043) as males' TTS during FATIGUE was higher (worse) than during CONTROL (p = 0.018). Finally, the model also revealed an effect of time × session type (F = 11.822, p < 0.001). TTS was higher (worse) POST FATIGUE compared to POST CONTROL (p < 0.001) and PRE FATIGUE (p < 0.001).

Time to stabilization pre and post a control and fatigue session for males (blue) and females (pink)



Pearson correlations demonstrating the relation between proximal hip strength and TTS are presented in Table 10 and Figures 24, 25, and 26. No significant correlations were identified, suggesting that hip strength and TTS following a BSLJL were unrelated.

Table 10

Pearson correlation coefficients illustrating the relation between proximal hip strength and time to stabilization when rested (CONTROL) and fatigued (FATIGUE) as well as the deficit in time to stabilization caused by fatigue

	Abduction	Abduction	External	External
	Force	Torque	Rotation Force	Rotation Torque
CONTROL	-0.061	-0.081	-0.021	0.002
FATIGUE	0.110	-0.125	0.121	0.173
FATIGUE Deficit	0.277	0.328	-0.180	-0.211

*significant at p < 0.05; **significant at p < 0.01.
Correlations between time to stabilization and proximal hip strength when in a rested state. Blue line: least squares regression; grey ribbon: 95% confidence interval



Correlations between time to stabilization and proximal hip strength when in a fatigued state. Blue line: least squares regression; grey ribbon: 95% confidence interval



Correlations between the change in time to stabilization caused by fatigue and proximal hip strength. A negative time to stabilization indicates fatigue worsened time to stabilization and a positive time to stabilization indicates it improved after fatigue. Blue line: least squares regression; grey ribbon: 95% confidence interval



Histogram and normal Q-Q plots for DPSI are presented in Figure 27. TTS is not normally distributed and appears positively skewed, indicating that most trials are relatively low (better) with less frequent higher (worse) trials. Boxplots presenting the median, quartiles, minimum, and maximum and means with 95% confidence intervals are presented in Figure 28 and 29.





Boxplot and mean and 95% confidence interval for dynamic postural stability index for males (blue) and females (pink) before (PRE) and after (POST) a control session and two fatigue sessions on the dominant and non-dominant leg



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The linear mixed-effects model evaluating DPSI revealed an effect of session type (F = 22.139, p < 0.001) as DPSI was higher (worse) during FATIGUE than CONTROL. There were no other significant effects identified (Figure 29).

Dynamic postural stability index pre and post a control and fatigue session for males (blue) and females (pink)



Pearson correlations demonstrating the relation between proximal hip strength and DPSI are presented in Table 11 and Figures 30, 31, and 32. No significant correlations were identified, suggesting that hip strength and TTS following a BSLJL were unrelated.

Table 11

Pearson correlation coefficients illustrating the relation between proximal hip strength and time to stabilization when rested (CONTROL) and fatigued (FATIGUE) as well as the deficit in time to stabilization caused by fatigue

	Abduction	Abduction	External	External
	Force	Torque	Rotation Force	Rotation Torque
CONTROL	-0.432*	-0.335	-0.208	-0.177
FATIGUE	-0.464*	-0.360	-0.157	-0.124
FATIGUE Deficit	0.451*	0.445*	0.160	0.127

*significant at p < 0.05; **significant at p < 0.01.









Correlations between the change in dynamic postural stability index following fatigue and proximal hip strength. A negative dynamic postural stability index indicated it worsened following fatigue and a positive dynamic postural stability index indicated it improved after fatigue. Blue line: least squares regression; grey ribbon: 95% confidence interval



6.4. Discussion

Characterizing the relations between indicators of ACL injury risk, including biological sex, fatigue, DPS, and proximal hip strength, will allow providers to empirically monitor injury risk with high sensitivity and strategically intervene prior to injury incidence. Based on previous investigations it was hypothesized that DPS would be compromised after completion of a short, high-intensity exercise bout (Bond et al., 2020; Shaw et al., 2008; Wikstrom et al., 2004) and that subjects with greater hip strength will demonstrate superior DPS (Bandholm et al., 2011; Lephart et al., 2002; Neamatallah et al., 2020; Suzuki et al., 2015; Williams et al., 2016; Zazulak et al., 2005).

From PRE to POST FATIGUE, TTS increased (worsened) for both males and females, with males potentially demonstrating greater fatigue related decrements compared to females; however, PRE to POST FATIGUE change was not observed for DPSI. In regards to difference in DPS between males and females, these findings are in agreement with previous studies (Dallinga et al., 2016; Ebben et al., 2010; Lephart et al., 2002; Wikstrom et al., 2006) that have demonstrated no clear difference between biological sexes; although, the present study and previous studies have used heterogeneous motor control tasks and DPS computational methodologies. Regarding the effect of fatigue on DPS, these findings are contrary to our hypothesis and in opposition to other studies that have used DPS to assess neuromuscular eccentric control following a single-legged jump-landing as decrements in both TTS and DPSI were anticipated. For example, Wikstrom et al. (2004) assessed 20 healthy college aged males and females and found that isokinetic and functional fatigue protocols caused similar decrements in DPS, Bond et al. (2020) evaluated six recreationally active high-school males and revealed that fatigue induced via long-duration sub-maximal intensity exercise tended to cause a decrement in DPS that was amplified in a hot and

humid environment compared to a temperate environment, and Shaw et al. (2008) appraised female collegiate volleyball players and found that DPS increased (worsened) following a shortterm functional fatigue protocol. Also of note, even though an approximate 0.30 s decrement in TTS was observed post-fatigue for the sample, this decrement may not be detectable in a single subject as the MDC exceeds 1.0 s (Chapter 5).

There are several potential reasons for the small or non-existent decrement in DS postfatigue. The short-term functional fatigue protocol used in the present study mimics the neuromuscular and physiological demands experience during athletic participation. In the present study, all subject's peak heart rate exceeded 85% of their predicted maximal heart rate before or during the fourth round of the fatigue protocol, which took approximately 5 to 7 minutes to complete. However, fatigue is a complex physiological phenomenon (Enoka & Duchateau, 2008; Enoka & Stuart, 1992). Fatigue has been defined as either central, which is related to gradual exercise induced reduction in the level of voluntary neuromuscular activation due to impairments proximal to the neuromuscular junction, or peripheral, which is related exercise induced processes leading to a reduction in the force-generating capacity of the muscle distal to the neuromuscular junction potentially due to metabolic or muscle damage related factors (McLean et al., 2007; Mclean & Samorezov, 2009). Although an elevated heart rate may be an indicator of central fatigue, its effect on voluntary neuromuscular activation in the present study is unknown. In a study to assess the effect of a fatigue protocol on voluntary neuromuscular activation, Mclean and Samorezov (2009) had subjects perform single-legged squats between trials of a forward singlelegged jump-landing and considered the subjects fatigued when they could no longer perform the squats unassisted, which would indicate reduced voluntary neuromuscular activation and fatigue. Non-functional fatigue protocols can also assess the reduction in voluntary neuromuscular

activation, such as the isokinetic fatigue protocol used by Wikstrom et al. (2004) which considered the subject fatigued when their peak torque dropped below 50% of the peak torque they displayed on the first repetition. Quantifying fatigue and its central and peripheral contributions further may give additional insights into the effect of fatigue on DPS, and it is possible that the fatigue protocol used here did not result in physiological or neuromuscular based decrements in DPS. Another potential reason for the small or nonexistent decrement in DPS post-fatigue is the rate of recovery following this particular fatigue protocol. Fox et al. (2008) demonstrated that fatigue induced decrements in static postural control were transient and started to return to baseline within minutes of completing the fatigue protocol. Therefore, despite initiating the BSLJL within 30 seconds of completing the fatigue protocol and completing all trials within 2 minutes of the completion of the fatigue protocol, it is possible that any delirious effects caused by fatigue were diminished within the first minute and were not captured by later BSLJL trials. In this regard, it is possible that a long duration or higher intensity fatigue protocol or the presence of additional factors such as hyperthermia or hypohydration (Bond et al., 2020; Distefano et al., 2013) are needed to more fully elucidate the effects of fatigue on DPS.

Also contrary to our hypothesis, proximal hip strength seems unrelated to DPS except for hip abduction strength and DPSI. It is possible that this relation was found in particular because DPSI captured poor DPS in all three planes, and proper strength and neuromuscular control of the hip abductors plays an important role in keep the pelvis level and centered over the base of support (Powers, 2010). Comparatively, TTS is mostly reflective of DPS in the vertical plane, so it is possible that hip extensor and knee extensor strength is more related to TTS. Nevertheless, although several studies have demonstrated that individuals with poor strength display aberrant biomechanics such as dynamic knee valgus and minimal knee flexion during a single-legged jumplanding that are associated with ACL injury risk (Bandholm et al., 2011; Neamatallah et al., 2020; Suzuki et al., 2015; Zazulak et al., 2005), it is possible that these aberrancies do not translate to diminished DPS. For example, Lephart et al. (2002) demonstrated that lower-extremity strength was unrelated to DPS, but did result in a straighter knee at initial contact and reduce knee flexion through the weight absorption phase following a single-legged jump-landing, particularly in females. Of note, about half of the subjects in the present study exceeded the clinical cutoff of 35.4% hip abduction peak force to body weight established by Khayambashi et al. (2016), but most subjects did not meet the clinical cutoff of 20.3% for hip external rotation.

In relation to the development of ACL injury risk screens, the lack of relation between proximal hip strength and DPS is remarkable and does not invalidate either as a risk factor. One reason that many ACL injury risk screens do not succeed is that they fail to explain sufficient variance between subjects, that is they do not have adequate discriminatory capacity to stratify an individual's ACL injury risk (Bahr, 2016). However, when two independent risk factors, that have both been found prospectively associated with ACL injury in separate studies (DuPrey et al., 2016; Khayambashi et al., 2016), are statistically unrelated, it means that they are explaining different proportions of the variance between individuals that would subsequently sustain and ACL injury and those that do not. In fact, the pursuit of multivariate injury risk screens, where the variables have little covariance with each other but have high between subject variance themselves, is critical as this leads to the most parsimonious and discriminative injury risk screen. Although it appears that greater results in less aberrant biomechanics (Bandholm et al., 2011; Lephart et al., 2002; Neamatallah et al., 2020; Suzuki et al., 2015; Williams et al., 2016; Zazulak et al., 2005) some research has demonstrated that strength training and subsequent increases in strength alone do not improvement biomechanics (Ford et al., 2015; Myer et al., 2005; Paterno et al., 2004). However,

when strength training programs are paired with neuromuscular training, which included plyometric, agility, and sport-specific drills designed to mimic the neuromuscular demands that athletes might face during athletic participation, an improvement in biomechanics is observed (Ford et al., 2015; Myer et al., 2005; Paterno et al., 2004).

There are several limitations of this study and possibilities for future research. The subjects in the present study represented a homogenous group of college aged, recreationally active subjects. Including a larger sample of adolescent and young adult subjects that represent a greater proportion of the 15 and 34 year of old age group who are competitive athletes, recreationally active, and sedentary would enhance the generalizability of these reliability findings. Further, this study excluded subjects with a history of significant unilateral pathology, such as an ACL injury and subsequent ACLR, but these subjects may display unique relations between DPS, hip strength, and fatigue. Further, the distance the subject started the BSLJL form the hurdle was not controlled for between subjects or within a subject between trials, which could affect the measurement of TTS and DPSI. Subjects were encouraged to refrain from strenuous activity 24 hours before study sessions; however, their activity level was not quantified or monitored, and it is possible that residual fatigue may have been present.

6.5. Conclusion

Poor DPS, quantified as TTS, following a BSLJL and proximal hip strength have been prospectively associated with non-contact ACL injury risk (DuPrey et al., 2016; Khayambashi et al., 2016). The simple and intuitive nature of DPS and hip strength assessments makes them ideal for wide-spread implementation as a multivariate ACL injury risk screen. Characterizing the relations between indicators of ACL injury risk, including biological sex, fatigue, DPS, and proximal hip strength, will allow providers to empirically monitor injury risk with high sensitivity

and strategically intervene prior to injury incidence. This study demonstrated that DPS when quantified as TTS was compromised by fatigue for both males and females, but DPS quantified as DPSI was not compromised. Further, hip abduction strength is associated with DPS when quantified as DPSI, indicating that individuals with greater hip abduction strength have superior DPSI. This information can be used to design multivariate ACL injury risk screens that have high discriminatory capacity.

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