SEX EFFECTS ON ANKLE BIOMECHANICS DURING SPORTS-RELEVANT TASKS AND PERONEAL MUSCLE PARAMETERS

By

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ABSTRACT

Introduction: Ankle sprains from excessive inversion are the most frequent sports-related injury. Common ankle prophylactics are designed to prevent injury by limiting excessive ankle inversion, yet may restrict other ankle motions leading to repeated re-injury. Females are twice as likely as males to suffer an ankle sprain, however, it is unknown if a sex dimorphism in ankle biomechanics exists when wearing ankle prophylactics, and whether differences in the peroneal musculature exist between sexes. **Purpose:** To quantify the ability of ankle prophylactics (Ankle Roll Guard (ARG), Brace, Control, and Tape) to prevent excessive ankle inversion during a sudden inversion event, and determine whether the effectiveness of the ankle prophylactics and in vivo peroneal muscle parameters differ between sexes. **Methods:** Thirty-two (16 male and 16 female) participants had dominant limb (i.e., braced) frontal and sagittal plane ankle biomechanics, including peak inversion and plantarflexion angle and range of motion (ROM), and time to peak inversion, quantified during the sudden inversion event with four prophylactic conditions (ARG, Brace, Control, and Tape) and peroneal muscle parameters recorded. With each prophylactic, participants performed five successful trials of the sudden inversion event. Peroneal muscle parameters, including physiological cross-sectional area (PCSA) and stiffness, were quantified in vivo using ultrasound shear-wave elastography, while peroneal strength was measured with an isokinetic dynamometer. **Statistical Analysis:** All kinematic variables were submitted to a RM ANOVA to test for main effect and interaction of brace (ARG, Brace, Control, and Tape) and sex (male and female). Peroneal muscle parameters were also submitted to independent samples t-test to test the effect of sex. **Results:** A prophylactic by sex interaction ($p = 0.010$), revealed females exhibit greater ankle inversion ROM with Control and ARG ($p = 0.001$, $p = 0.010$) compared to males. Females also exhibited greater ankle inversion ROM with ARG compared to Brace ($p = 0.001$), and Control compared to Brace and Tape ($p < 0.001$, $p < 0.001$), while males exhibited no significant difference between any prophylactic condition ($p > 0.05$). Ankle prophylactic impacted ankle inversion ROM ($p < 0.001$), time to peak inversion ($p < 0.001$), and peak plantarflexion angle ($p < 0.001$) and ROM ($p < 0.001$). Females exhibited smaller peroneal PCSA ($p = 0.002$) and dorsiflexion strength ($p = 0.047$), but sex had no significant effect on peroneal strength ($p =0.142$) or stiffness ($p > 0.05$). **Conclusion:** The protective benefits of ankle prophylactics may depend on the specific device and sex of the user. With the lace-up brace and tape, participants decreased ankle biomechanics associated with injury, but this protective benefit was only evident for females. Females exhibited a sex dimorphism in ankle biomechanics during the sudden inversion event, and smaller and weaker peroneals that may contribute to the sex disparity in injury rate.

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LIST OF ABBREVIATIONS

FAI	Functional Ankle Instability
SE	Shear Wave Elastography
3D	Three-Dimensional
Con	Control Subject Group
ARG	Ankle Roll Guard
GRF	Ground Reaction Force
BW	Body Weight
IC	Initial Contact
PS	Peak Stance
ROM	Range of Motion
CSA	Cross-Sectional Area
PCSA	Physiological Cross-Sectional Area

CHAPTER ONE: INTRODUCTION

Ankle sprains are the most common injury experienced during sport[1]. An ankle sprain accounts for 14% of all sports-related injuries and 3% of all emergency room visits[2]. Ankle sprain injuries have a substantial financial and physical cost for the injured. In 2003, the direct cost for treating sports-related ankle injuries alone exceeded $70 million dollars, with indirect costs of lost time at work or sports participation totaling $1.1 billion dollars[3,4]. But, the rate of ankle injury reportedly differs between males and females. A 2014 meta-analysis found females display almost twice the injury rate as males[5]. Females are reported to suffer 13.6 ankle sprains per 1000 athletic exposures compared to 6.94 ankle sprains per 1000 athletic exposures for their male counterparts[5].

Ankle injuries typically occur from significant frontal plane motion of the joint. Specifically, an ankle sprain occurs from excessive ankle inversion past 30 degrees [5–7]. As a result, ankle prophylactics have been designed to prevent excessive inversion and subsequent injury. The use of an ankle brace is documented to decrease peak ankle inversion by 5° and total inversion range of motion up to 12° compared to an un-braced ankle[8–10]. Similarly, it takes an individual 15% longer to reach peak ankle inversion when wearing an ankle brace[9], providing the associated musculature greater time to stabilize the joint and prevent injury. As a result, these braces reportedly produce a 69% reduction in ankle sprain in both elite and recreational athletes[11]. Despite their reported success, ankle braces may not prevent re-injury of the joint. Approximately 20% of individuals experienced re-injury of the ankle within 6 to 18 months, despite prolonged bracing with a prophylactic[12]. The prolonged ankle bracing reportedly contributes to reduced functional capacity of the joint[13]. The compromised ankle joint function results in decreased joint range of motion and increased neuromuscular dysfunction of the peroneal musculature that negatively effects performance and may lead to re-injury[13,14]. These deficits commonly lead to the onset of functional ankle instability (FAI), or repeated “giving way” of the joint. FAI occurs from reoccurring episodes of excessive inversion and often leads to of significant deterioration of the ankle later in life[15–17]. A sex dimorphism in FAI is also apparent[18]. The incidence of FAI is approximately 32% more prevalent in females than their male counterparts[19]. However, the reason for the increased prevalence of FAI development in females is largely unknown and may stem from neuromuscular differences of the peroneal musculature exhibited between sexes or bracing that is ineffective for females.

A sex dimorphism in ankle neuromechanics (i.e., joint biomechanics and neuromuscular control) may contribute to FAI. During sports-relevant tasks, females exhibit greater frontal[20,21] and sagittal plane[20,22] ankle motions, which are thought to contribute to their increased injury risk[23,24]. Reportedly, females exhibit up to 10^{o} more ankle dorsiflexion and 4^{o} more ankle inversion during sports-relevant tasks than their male counterparts[20,21]. These differences in biomechanics may stem from a dimorphism in ankle neuromuscular control between males and females. Neuromuscular control provides dynamic restraint of the joint, i.e., stability, through preparatory, unconscious activation of the muscle in order to provide functional joint stability[25]. Females have been shown to exhibit greater preparatory peroneal muscle activation during sports-relevant tasks than male participants[26,27]. This increased peroneal neuromuscular activation may protect and stabilize the ankle during weight bearing activities, but stem from differences in muscle strength and size between sexes. Females reportedly exhibit a 32 to 39% reduction in maximum peroneal strength, as well as significantly smaller cross sectional area of lower limb musculature compared to males[28–30]. However, to our knowledge, it is unknown whether similar sex differences exist in the peroneal musculature that is thought to prevent ankle injury. Recently ultrasound technology has improved researchers' ability to measure *in vivo* muscle parameters. Most importantly, the recent development of shear wave elastography (SE) provides researchers the ability to measure *in vivo* muscle stiffness. Muscle stiffness plays an important role in performance and injury. While muscle stiffness is necessary for optimal levels of performance, high levels of muscle stiffness reportedly relate to bony injuries and low levels stiffness to soft tissue injuries[31]. In fact, individuals suffering from medial tibial stress syndrome exhibit greater stiffness of shank musculature (i.e., tibialis anterior, gastrocnemius, and soleus) than healthy controls.[32] SE measurements may provide a more accurate estimation of individual muscle force than quantifying neuromuscular activation with surface electromyography devices[33], using ultrasound technology to asses peroneal stiffness may provide a more accurate estimation of muscle function. It is currently unknown if *in vivo* parameters of the peroneal musculature differ between sexes, or whether stiffness of this musculature impacts ankle biomechanics related to injury of the joint, particularly during sports-relevant tasks.

Specific Aims

Specific Aim 1:

To examine ankle kinematics during a sudden inversion event to determine if joint biomechanics differ between sexes.

Hypothesis:

A significant sex dimorphism in ankle biomechanics will be evident during the sudden inversion.

Subhypothesis 1: During the sudden inversion event, females will exhibit significantly greater peak ankle inversion angle, total ankle inversion range of motion, and faster time to peak ankle inversion compared to male counterparts.

Subhypothesis 2: During the sudden inversion event, females will exhibit significantly greater peak ankle plantarflexion and ankle plantarflexion ROM compared to their male counterparts.

Significance:

There is a lack of existing literature that directly compares ankle biomechanics between male and female participants during a sudden inversion event. The knowledge regarding ankle function during a sudden inversion event can help determine the etiology of ankle sprain and the biomechanics that lead to the sex disparity injury rate, which will potentially lead to the development of sex-specific injury prevention, treatment and rehabilitation methods, as well as sex-specific prophylactics.

Specific Aim 2:

To quantify the ability of conventional prophylactics to prevent excessive ankle inversion for males and females during a sudden inversion event. We will quantify ankle kinematics, specifically ankle inversion and plantarflexion, with and without an ankle prophylactic to determine the effectiveness of these devices to prevent ankle motions that lead to injury for both sexes.

Hypothesis:

When wearing an ankle prophylactic, participants will exhibit altered ankle kinematics during the sudden inversion event, but male participants will exhibit greater alterations than their female counterparts.

Subhypothesis 1: During the sudden inversion event, when wearing an ankle prophylactic participants will exhibit a significant reduction in peak ankle plantarflexion and inversion, as well as smaller ankle plantarflexion and inversion range of motion compared to a control condition.

Subhypothesis 2: During the sudden inversion event, male participants will exhibit a greater reduction in peak inversion and inversion range of motion while wearing an ankle prophylactic compared to their female counterparts.

Significance

Understanding the effectiveness of ankle braces to prevent excessive joint inversion will provide insight to their ability to prevent initial sprain and re-injury, as well as determine their effectiveness to prevent injury for both sexes. Determining whether ankle motions differ between prophylactic devices may provide fundamental information into whether sustained use leads to functional performance deficits that cause functional ankle instability. As a result, this information can be implemented by health care professionals during treatment and rehabilitation protocols to reduce the likelihood of the development of functional instability at the ankle.

Specific Aim 3:

To compare *in vivo* peroneal muscle parameters between male and female participants. Specifically, the study will quantify physiological cross-sectional area (PCSA), maximum isometric strength, and peroneal muscle stiffness to determine whether these parameters differ between sexes.

Hypothesis:

Females will exhibit a smaller, weaker, and stiffer peroneal than their male counterparts.

Subhypothesis 1: The female participants will exhibit a significantly smaller PCSA of the peroneal musculature than their male counterparts.

Subhypothesis 2: The female participants will exhibit a significantly smaller maximum plantarflexion, dorsiflexion, inversion and eversion strength than their male counterparts.

Subhypothesis 3: The female participants will exhibit a significant increase in the stiffness of the peroneal musculature compared to their male counterparts.

Significance:

Knowledge of whether the peroneal musculature differs between sexes can provide insight into sex disparity of ankle sprain and development of functional ankle instability. The use of novel ultrasound technology allows for direct measurement of *in vivo* peroneal muscle parameters that may provide fundamental knowledge regarding sex dimorphism in ankle biomechanics and injury. As a result, both researchers and clinicians can accurately measure parameters of the peroneal musculature that can be used to tailor injury prevention and treatment protocols to specific individuals.

CHAPTER TWO: LITERATURE REVIEW

The following section aims to detail ankle sprains, specifically the 1) incidence of ankle sprains across populations, 2) anatomy of ankle sprains, 3) development of ankle prophylactic braces as treatment for ankle sprain, 4) ankle brace consequences and the development of functional ankle instability, 5) sex dimorphism in ankle biomechanics, and 6) recent advances in ultrasound technology to help identify risk factors for ankle sprain.

Ankle Sprain Epidemiology

Historically, ankle sprains are the most common recreational injury[1]. Ankle sprains result in 14% of sports related injuries and 3% of all emergency room visits[2]. These injuries have significant physical and economic costs. It has been shown that after an ankle sprain, 80.7% of individuals used ice treatment, 55.4% utilized crutches, and 56.6% took non-steroidal anti-inflammatory drugs (NSAIDs)[2]. Furthermore, after injury over 6% of individuals could not maintain their previous occupational activities[34]. Absence from work or recreation has significant economic costs as well. In 2003, the direct cost for treating sports-related ankle injuries alone exceeded $70 million dollars, with indirect costs of lost time at work or sports participation totaled $1.1 billion dollars[3,4]. More recently, the median cost for treatment of a lateral ankle sprain was estimated at $1029 per patient[35].

Sex discrepancies of ankle sprain incidence have also been noted. A 2014 meta-analysis found females exhibit twice the injury rate as males[5]. Specifically, females suffer 13.6 ankle sprains per 1000 athletic exposures compared to 6.94 ankle sprains per 1000 athletic exposures exhibited by their male counterparts[5]. Because the most common risk factor for spraining your ankle is having a history of at least one ankle sprain, a higher incidence of ankle sprain in females puts them at greater risk for repeated injury[36]. But, to date, it is largely unknown why females exhibit greater incidence than males.

Ankle Anatomy

The high incidence and debilitation of ankle sprain may be a result of the joint's unique anatomy. The ankle complex is made up of 3 articulations; the talucrural joint, the distal tibiofibular syndesmosis, and subtalar joint, all of which assist in stabilization and movement of the ankle. Specifically, the talucrural joint acts to transmit force during stance from the lower leg to the foot[37]. Soft tissue surrounding the talocrural joint includes several supportive ligaments, including the calcaneofibular (CFL), the posterior talofibular (PTFL), and the anterior talofibular (ATFL). The ATFL has been shown *in vitro* to primarily prevent anterior displacement of the talus and excessive inversion of the ankle[37]. However, compared to the PTFL and CFL, the ATFL is the weakest ankle ligament, and therefore the most commonly injured[37–39]. As a result, ankle sprains most commonly occur from excessive inversion[40]. The ankle has an average inversion range of motion (ROM) between 20 to 30°, and injury is thought to occur when inversion motions significantly exceed the 30° threshold[24]. This has led to an increasing research focus on healthcare solutions to prevent excessive ankle inversion and subsequent injury.

Ankle Prophylactic Braces

Due to readiness and cost-efficiency, ankle prophylactics have primarily been used as a means for preventing ankle sprains. Ankle prophylactics most commonly consists of a lace-up brace or non-elastic tape wrapped around the joint to restrict range of motion[41]. Non-elastic tape is applied in a closed-basket weave pattern, where the ankle is held at 90 degrees of dorsiflexion as lateral anchor stirrups and figure eights are applied around the joint. Lace-up braces are generally classified as being made of cloth or nylon and include two Velcro stirrup straps and laces similar to a high-top shoe. Case study data has shown out of 13,500 athletic exposures only one injury occurred while wearing an ankle prophylactic[42]. Similarly, a 69% reduction in ankle sprain was displayed with the use of an ankle brace[11]. A possible rationale for ankle prophylactics' ability to reduce ankle sprain incidence is their ability to increase kinesthetic awareness and limit ankle inversion motion[11]. In order to justify the effectiveness of these ankle prophylactics, researchers have observed biomechanical performance measures during a variety of sports-relevant tasks.

Ankle Prophylactic Biomechanics

Sudden Inversion Event

To examine the effectiveness of ankle prophylactics researchers have simulated ankle sprain motions through a sudden inversion event. During a sudden inversion event, participants stand with feet shoulder width apart on a wooden platform that contains two trap doors. With the use of a trigger system, one of the doors is released to induce ankle inversion. These events produce approximately 30° of inversion, which has been deemed safe and ethical for participants[43]. During a sudden inversion event, non-elastic ankle tape has been shown to produce 10^o less inversion range of motion (ROM)[8,9,44] and 12^o lower peak ankle inversion angle than an unbraced ankle[45]. Furthermore, ankle tape has exhibited 15% slower time to peak inversion[9]. A decrease in the inversion rate and ROM observed with ankle tape can allow for reflex mechanisms of the body to respond and potentially prevent or lessen ankle sprain severity[9]. Lace-up style prophylactics may provide similar restriction of ankle inversion, as a lace-up brace reportedly exhibit approximately 8^o lower peak inversion angle and 23^o less inversion ROM compared to an unbraced ankle during a sudden inversion event[46]. Additionally, lace-up braces exhibited decreased inversion velocity by 35% during a sudden inversion event[47]. When compared directly to non-elastic ankle tape, no significant difference in ankle inversion between prophylactics was evident[47]. This indicates that lace-up braces may prevent ankle sprain to a similar capacity as ankle tape. While a sudden inversion event allows for direct quantification of ankle prophylactics' ability to reduce hazardous ankle biomechanics and subsequent injury, ankle sprains commonly occur during dynamic movement. Thus, dynamic movements more closely related to sports-specific tasks are recommended to fully observe ankle prophylactic ability to prevent injury[48].

Drop Landing

Though ankle prophylactics reduce harmful excessive ankle inversion, they may also reduce other necessary ankle motions (i.e, plantarflexion and dorsiflexion) during sports-relevant tasks. In particular, landing has been deemed a highly important dynamic movement during athletic activity in which the ankle joint provides crucial energy absorbtion[49]. Ankle tape has been shown to reduce ankle sagittal plane ROM up to 18% during one and two legged drop landings compared to a unbraced condition[50,51].

Similarly, lace-up braces exhibited 10% to 33% reduction of ankle sagittal plane ROM during drop landing[52,53]. The reduced ankle sagittal plane ROM exhibited with ankle prophylactics may impact the body's ability to effectively dissipate ground reaction forces during landing. Ankle tape and lace-up braces have displayed 10% to 13% greater peak vertical ground reaction force compared to a unbraced condition during a drop landing task[52,54]. The larger ground reaction forces observed with ankle prophylactic use may increase risk of musculoskeletal injury during drop landing[55]. Lace-up braces however, may greater alter the body's ability to dissipate ground reaction forces, as time to peak vertical ground reaction force was two times faster with a lace-up brace compared to tape[56]. A higher rate of force that is applied to the body may increase risk of musculoskeletal injury from the rapid impact sent to the lower limb[51]. Thus, the impact of ankle prophylactics' on deleterious biomechanics during drop landing may differ between prophylactic design and warrant future research.

Vertical Jump

The reduced ankle sagittal plane motion displayed by ankle prophylactics may also impact sport performance. Ankle tape and lace-up braces reportedly decrease jump height 2 cm during a countermovement jump compared to a control condition[57–61,62]. This reduction in performance may be associated with an inability to produce high ground reaction forces with restricted ankle motions, as decreased plantarflexion angle during vertical jump has been correlated to reduced jump height and peak ankle work[60,63]. In a dynamic, explosive activity such as a vertical jump, reduced joint motions with an ankle prophylactic may be detrimental to the success of movement. However, kinetic data during a vertical jump with an ankle prophylactic remains largely unavailable from the literature. Future research should look to observe kinetic data as well as jump height measurements in an attempt to further analyze the impact of ankle prophylactics during a maximum vertical jump.

Walking and Running Gait

Similar performance deficits with ankle prophylactic use may be evident during gait. Ankle tape and lace-up braces have exhibited a 2^{o} to 5^{o} reduction in peak inversion angle and 5^{o} to 9^{o} reduction of inversion ROM compared to a control condition during an over-ground running task[64,65]. While decreased ankle inversion is beneficial to lowering risk of ankle sprain, it may also illustrate reduced joint capacity and negatively impact performance. Ankle tape reportedly reduced plantarflexion ROM approximately 36% during a running task[64,65]. This reduction in sagittal plane motion with ankle tape during gait may negatively impact running performance, as the use of ankle tape also exhibited a 5% increase in energy expenditure[65]. However, the impact of ankle tape during gait can change with continuous exercise. Effects of ankle tape have been shown to no longer significantly impact ankle kinematics after 20 to 30 minutes of continuous exercise[64–66]. Further, contrary to ankle tape, using a lace-up brace did not alter sagittal or frontal plane ROM or have significant impact on energy exposure during 30 minutes of continuous gait[65,67]. Thus, lace-up braces may not significantly impact performance during gait. Future research is warranted to observe ankle prophylactic impact on user performance during sports-relevant tasks and determine if deleterious biomechanics are exhibited with prophylactic use and differ by design.

Ankle Brace Consequences and Functional Ankle Instability

While ankle prophylactics have been shown to limit excessive inversion and subsequent injury risk, they also restrict other ankle motions that may impact the body's ability to prevent re-injury of the ankle. Approximately 20% of individuals experienced re-injury of the ankle within 6 to 18 months, despite prolonged bracing with a prophylactic[12]. Prolonged use of ankle prophylactics reportedly contribute to reduced functional capacity of the joint[13]. This reduction of ankle joint function results in decreased joint range of motion and increased neuromuscular dysfunction of the peroneal musculature that negatively affects performance[13,14]. These deficits have the ability to lead to re-injury of the ankle joint and ultimately the development of functional ankle instability (FAI). FAI is defined by the sensation of the ankle "giving way" after the diagnosis of an inversion ankle sprain[15]. It has been estimated that 30% of individuals will develop FAI following an initial ankle sprain[68]. Furthermore, a sex dimorphism in FAI is also apparent[18], as the incidence of females developing FAI is reportedly 32% higher than male counterparts[18].

Sex Differences Ankle Biomechanics

Unbraced Condition

A higher rate of FAI development in females may be affected by sex dimorphism in ankle biomechanics. Females have shown 3^{o} larger peak inversion angle[21] and approximately 20^{o} more inversion ROM[20] during a drop landing than their male counterparts. Moreover, females have exhibited 10^{o} to 17^{o} more ankle plantarflexion ROM during landing[20,22]. Similar trends exist when observing sex difference during other sports-relevant tasks. Females have also exhibited 4^{o} higher peak inversion angle and

13% greater relative ankle joint work than male counterparts during a side-step cutting task[21]. This indicates that increased range of motion in both the frontal and sagittal plane about the ankle observed in females may increase their likelihood of ankle sprain in an unbraced condition[23,24]. Further, increased ankle work in females may indicate a greater amount of muscular effort about the joint to slow down the body's center of mass to prevent ankle sprain. However, differences in ankle biomechanics between males and females during sports-relevant tasks remains largely unknown and future research is warranted to observe sex dimorphism in ankle biomechanics and impact on ankle sprain injury risk.

Braced Condition

Females' augmented ankle range of motion may also impact effectiveness of ankle prophylactics. According to Niu et al.[69], the use of ankle prophylactics may contribute to increased dynamic frontal plane instability in females and subsequent risk of ankle sprain injury. Females have displayed a 33% to 54% greater medial-lateral ground reaction force with ankle tape and lace-up brace during a drop landing compared to male counterparts[69]. However, due to the small contribution of medial-lateral ground reaction force to overall ground reaction force, the extent of the clinical significance of these results is unknown. Further, sex differences with ankle prophylactics have also been observed during maximum vertical jump. Females reportedly showed a 3% greater decrease than males in vertical jump height with the use of an ankle tape compared to an untaped condition[57]. This indicates that the use of ankle prophylactics may cause greater functional and sport-performance deficits in females than in males. Future research should look to observe if a sex dimorphism is evident in ankle biomechanics during sports-relevant tasks with prophylactic use.

Peroneal Musculature

Differences in ankle biomechanics between males and females may stem from a sex dimorphism in ankle neuromuscular control. Neuromuscular control aids in dynamic restraint and stability of the joint through preparatory, unconscious activation of the muscle[25]. At the ankle, the primary muscles used for maintaining foot position through neuromuscular control during physical activity are the peroneus longus and peroneus brevis, making up the peroneal musculature[14]. Neuromuscular dysfunction of the peroneal musculature can cause instability about the ankle and ultimately lead to injury[14]. Females have been shown to exhibit approximately 12% greater and 7% longer peroneal muscle activation during gait than male counterparts[26,27]. Thus, the activation strategies of ankle musculature, in particular the peroneal, may differ between sexes and increased peroneal muscle activation exhibited by female participants during stance phase may be needed to aid in the decreased dynamic posture control females display[70]. Additionally, the increased peroneal activation may be a result of sex dimorphism in muscle size and strength. Females reportedly exhibit a 32 to 39% reduction in maximum peroneal strength compared to males[29]. The reduction in muscular strength about the ankle may indicate need for greater peroneal activation to stabilize the joint in an attempt to prevent ankle sprain. Conversely, when data was normalized to participant weight, no significant difference in maximum peroneal strength was displayed[29]. Thus, sex differences in muscular strength may be directly linked to common discrepancies in size between males and females. To our knowledge however, the extent of sex dimorphism in ankle musculature and impact on ankle sprain injury risk is largely unknown.

Ultrasound Technology

Recently, ultrasound technology has improved researchers' ability to take quantitative measurements and observe mechanical properties of muscle tissue *in vivo.* Through the use of ultrasound sonography, researchers are now able to measure various muscle parameters such as pennation angle, fascicle length, cross sectional area, and muscle thickness. Males have exhibited 7% larger muscle thickness and 10% greater angle of pennation in lower limb muscles than female counterparts[71]. This incongruity may have a significant impact on muscle force and velocity. A larger pennation angle permits a greater number of muscle fibers per cross-sectional area and results in a larger overall muscle force with the same volume of muscle[71,72]. The ability to produce a larger muscle force by males may apply to the peroneal musculature, and thus lower risk of ankle sprain. However, sex discrepancy in muscle thickness and pennation angle for the peroneal musculature is largely unknown.

Males also display a 14% larger lower limb muscle cross-sectional area (CSA) than their female counterparts[30]. Similar to pennation angle and muscle thickness, a larger cross-sectional area allows greater muscle force with the same tendon attachment[71]. Individuals who have suffered a lateral ankle sprain exhibit a smaller peroneal CSA compared to healthy controls[73]. Thus, females' generally smaller muscle CSA compared to males may increase risk of ankle sprain. CSA has limitations when comparing groups with contrasting muscle architecture[74]. A more accurate measurement may be the use of physiological cross-sectional area (PCSA). PCSA is the measurement of muscle cross-sectional area at right angles to the longitudinal axis of the muscle fibers, while also taking into account the total muscle volume and length of the fibers[74]. Thus, ultrasound sonography PCSA measurements allow for direct quantification of the force-production capacity of *in vivo* skeletal muscle while taking into account confounding muscle architecture differences. To date, however, it is largely unknown if PCSA measurements of the peroneal musculature differ between sexes.

Another development of ultrasound technology utilized in research is shear wave muscle elastography. Shear wave elastography is a novel way to measure stiffness of a particular muscle. This ultrasound technique submits a low frequency sonography to apply an acoustic compression force (stress) on the muscle tissue, causing an axial displacement (strain)[76]. Using Hooke's law, researchers calculate Young's elastic modulus of the muscle to quantify stiffness[77]. Shear wave elastography may provide a more accurate estimation of muscle stiffness than quantifying neuromuscular activation through conventional methods such as surface electromyography or muscle palpation [33]. Muscle stiffness plays an important role in performance and injury. Some level of muscle stiffness is reportedly necessary for optimal performance, as increased stiffness has been associated with greater running economy[31,78]. When observing muscle stiffness with respect to injury, increased stiffness reportedly relates to bony injuries and decreased stiffness with soft tissue injuries[31]. This has been shown to be evident when observing shear wave elastography measurements in individuals suffering from medial tibial stress syndrome (MTSS). Reportedly, MTSS participants exhibit approximately 11% higher tibialis anterior, gastrocnemius, and soleus muscle stiffness than uninjured controls[32]. This trend may carry over to the peroneal musculature, where higher muscle stiffness measurements indicate greater risk for ankle sprain. However, an optimal level of muscle stiffness for injury-free performance is largely unknown.

Limited research has also observed shear wave elastography measurements between males and females. One study has found no sex effect on active muscle stiffness of the tibialis anterior[79]. This indicates that muscle stiffness may not influence sex discrepancies in performance and injury. However, females in the study did display, albeit insignificant, a 3 to 14 kilopascals (kPa) greater shear modulus measurements than male counterparts[79]. Further, when observing upper body musculature, females display significantly higher muscle stiffness than male counterparts[80]. A trend of increased muscle stiffness in females may carry over to the peroneal musculature and increase their risk of ankle sprain injury. Yet, to date, it is largely unknown if sex impacts muscle stiffness of the peroneal musculature or if abnormal peroneal muscle stiffness alters ankle biomechanics that increase the risk of ankle sprain. Future research should quantify sex differences in *in vivo* peroneal muscle parameters using ultrasound sonography and shear wave elastography, as well as determine how these parameters impact ankle biomechanics.

Ankle sprain from excessive joint inversion is a frequent recreational injury, particularly for females. Numerous prophylactic devices have been developed to prevent initial and re-injury of the ankle. Research has demonstrated that these braces are effective at limiting inversion, but negatively impact performance and may not prevent re-injury. To date, limited data exists on the ability of these braces to prevent ankle biomechanics related to injury for both males and females. Considering ankle biomechanics reportedly differ between sexes during sports-relevant tasks, a sex dimorphism in ankle neuromuscular control may contribute to the high rate of ankle sprain for females. With the recent enhancement of ultrasound shear wave elastography, researchers can accurately assess *in vivo* peroneal muscle parameters for both sexes and determine the impact of peroneal neuromuscular function on ankle biomechanics. This information can be used to improve the effectiveness of ankle prophylactics for both males and females, and ultimately reduce the number of sports-related injuries.

CHAPTER THREE: MANUSCRIPT

Introduction

Ankle sprains are a common, costly sports-related injury[1,3,4]. These injuries typically occur from excessive ankle inversion motion past 30 degrees[5–7]. As a result, ankle prophylactics have been designed to prevent injury by restricting ankle inversion[13]. Common ankle prophylactics, such as lace-up braces and non-elastic tape, effectively reduce incidence of ankle sprain[11] by restricting peak ankle inversion angle and range of motion (ROM), as well as time to peak ankle inversion compared to an un-braced ankle during a sudden inversion event[8–10]. But despite their reported success, common ankle prophylactics may not effectively prevent re-injury of the joint, as approximately 20% of individuals experience ankle re-injury within 6 to 18 months of initial injury[12].

Prolonged ankle bracing reportedly contributes to reduced functional capacity of the joint[13] and peroneal neuromuscular dysfunction that leads to re-injury[13,14]. These deficits are commonly present at the onset of functional ankle instability (FAI), or reoccurring episodes of excessive joint inversion[15–17]. Development of FAI may result from common ankle prophylactics' restriction of joint plantar- and dorsi-flexion motions[51,52]. The deleterious effects of common ankle prophylactics motivated the development of the Ankle Roll Guard (ARG). The ARG is a novel ankle prophylactic that supposedly allows the user normal plantar- and dorsi-flexion motions while adding mechanical stability needed to prevent excessive inversion and reduce the likelihood of injury. Yet, effectiveness of the ARG compared to common ankle prophylactics (lace-up brace and tape) is currently unknown.

Females are twice as likely to suffer an ankle sprain than their male counterparts[5]. However, the reason for sex dimorphism in ankle injury is largely unknown, but may stem from ankle neuromuscular differences between the sexes. During sports-relevant tasks, females exhibit greater ankle plantar- and dorsi-flexion[20,22] and inversion[21] motions, biomechanics thought to increase injury risk[23,24], compared to males. The sex differences in ankle biomechanics may result from a dimorphism in neuromuscular control of the joint between males and females. Neuromuscular control provides dynamic joint restraint and functional stability through preparatory, unconscious activation of the associated musculature[25], such as the peroneals at the ankle. Females exhibit greater peroneal muscle activation during sports-relevant tasks than males[26,27]. The increased peroneal activation may be necessary for females to adequately protect and stabilize the ankle during weight bearing activities, because of their deficits in muscle strength and size compared to males. Females are typically smaller and weaker than their male counterparts[30,81], and in fact, exhibit between 32 to 39% reduction in maximum peroneal strength compared to males[29]. Although, females are reported to have significantly smaller cross sectional area of lower limb musculature compared to males[81], it is currently unknown whether sex differences in the peroneal musculature exist.

Recent advances in ultrasound technology have improved researchers' ability to measure *in vivo* muscle parameters. Most importantly, the recent development of shear wave elastography (SE) provides researchers the ability to measure *in vivo* muscle stiffness. Muscle stiffness plays an important role in performance and injury[31,76], and optimal muscle stiffness is necessary to prevent injury. Increased muscle stiffness reportedly relates to bony injuries and decreased stiffness to soft tissue injuries[31]. Using ultrasound sonography and SE measurements to assess peroneal stiffness may provide a more accurate estimation of muscle function[33] and identify potential neuromuscular risk factors of ankle sprain. Yet, it is currently unknown if *in vivo* parameters of the peroneal musculature differ between sexes. With that in mind, the primary purpose of this study was to quantify the ability of ankle prophylactics (ARG, Brace, Control, and Tape) to prevent excessive ankle inversion during a sudden inversion event, and determine whether the effectiveness of the ankle prophylactic differs between sexes. It is hypothesized that each ankle prophylactic will reduce ankle inversion and plantarflexion motions, as well as time to peak ankle inversion compared to the control condition. But, females would exhibit greater ankle plantarflexion and inversion motions, and faster time to peak ankle inversion with all prophylactic conditions compared to males. A secondary purpose of this study is to compare *in vivo* peroneal muscle parameters between male and female participants. It is hypothesized that females will exhibit a smaller, weaker, and stiffer peroneal than their male counterparts.

Methods

Subjects

An *a priori* power analysis of preliminary ankle inversion exhibited during a sudden inversion event indicated a minimum of 11 participants per group (sex) are needed to achieve 80% statistical power with an alpha level of 0.05. Thus, to ensure adequate sample size, we recruited thirty-two (16 male and 16 female) participants between the ages 18 to 30 years of age (Table 3.1). To be included, all participants must be in good health and recreationally active, as defined by a score of 5 or higher on the Tegnar scale (Appendix A)[82]. Potential participants were excluded if they had: (1) any pain or current symptoms of ankle sprain; (2) a history of fracture or surgery in the lower limbs; (3) and/or any known neurological disorders. Participants were further defined as functionally instable (FAI) or control (Con) based on their answers to the Ankle Instability Instrument (Appendix B)[16]. For this study, FAI participants indicated they have a history of at least one medically diagnosed ankle sprain accompanied by frequent sensations of "giving way"[15]; whereas, Con indicated no history of ankle sprain or "giving way". We attempted to recruit equal numbers of FAI and Con participants.

Table 3.1 Subject demographics mean (SD)

	N	Height (m)	Weight (kg)	Age (years)
Males	16	1.76 (0.04)	80.36 (9.57)	21.93 (3.13)
Females	16	1.68 (0.06)	64.59 (7.71)	21.40 (2.82)

Experimental Design

The study utilized a repeated measures design. Each participant performed two test sessions. Each test session lasted approximately 1.5 to 2 hours. During each session, participants performed all sports-relevant tasks with two ankle braces. The ankle braces included: ARG prophylactic brace (Armor1, Ankle Roll Guard, LLC, Boise, ID, USA), lace-up brace (ASO Ankle Stabilizer, MedSpec, Charlotte, NC, USA), closed basket weave ankle tape application, and unbraced control (Figure 3.1). To avoid bias and confounding data, a 4 x 4 Williams Design Latin square approach was used to randomize the testing order of the braces (Table 3.2).

Figure 3.1 Depiction of (a) Ankle Roll Guard (ARG), (b) ASO Ankle Stabilizer (Brace), and (c) closed-basket weave ankle tape (Tape).

Table 3.2 The Latin Square design used for randomization of the testing order for each brace condition.

	SESSION 1	SESSION 2	SESSION 3	SESSION 4
Order 1	ARG	Control	Brace	Tape
Order 2	Control	Tape	ARG	Brace
Order 3	Tape	Brace	Control	ARG
Order 4	Brace	ARG	Tape	Control

Peroneal Muscle Testing

At the beginning of testing, each participant had their basic anthropometric information, including height (m), weight (kg), and age (years) recorded. Next, each participant had strength, and *in vivo* parameters of the peroneal musculature measured for either the affected (medically diagnosed ankle sprain) limb (FAI) or dominant limb (Con). The affected limb was analyzed for FAI participants, as greater neuromuscular deficits are reported to occur following ankle sprain[83,84]. Participants self-reported leg dominance as the leg they preferred for kicking a soccer ball[85]. To quantify ankle strength, participants performed three maximum isometric dorsiflexion and plantarflexion, and inversion and eversion contractions on an isokinetic dynamometer (System 2, Biodex Medical Systems Inc, Shirley, NY). To quantify *in vivo* peroneal muscle parameters, each participant had three elastograms of their peroneal musculature recorded using an ultrasound 9L4 transducer (Siemens Acuson S2000). An elastogram was recorded while each participant (1) laid on their contralateral side, (2) stood shoulder width apart, and (3) stood on a wooden platform with their ankle inverted to 30 degrees. Participant foot position at 90^{o} of dorsiflexion was maintained during prone and standing elastograms. All *in vivo* muscle parameters were measured in using OsiriX (Pimeo SARL, Bernex, Switzerland). From each elastogram, muscle stiffness, i.e., shear modulus, were calculated according to Eby et al.[86] using the following formula:

$$G = c_s$$

where G is shear modulus, c_s is shear wave propagation velocity, and ρ is density which is assumed to be 1000 kg/m^2 for all skeletal muscle. Muscle volume was estimated according to Fukunaga et al.[87] using the following formula:

$$MV\ (m^3) = L \times (MT^2)$$

where MT is muscle thickness and L is leg length. Muscle volume was normalized to height and weight of all participants according Feger et al.[88]. Physiological cross sectional area (PCSA) was quantified according to Ward et al.[89] using the following formula:

$$PCSA = (MV\ (m^3) \times \cos(\theta)) / Lf\ (cm)$$

where θ is pennation angle and Lf is fiber length. Pennation angle and fiber length was measured from the peroneus brevis only due to limitations in the ability to visualize muscle fibers of the peroneus longus in vivo. PCSA was reported two ways: (1) normalized to standard muscle volume and (2) normalized to muscle volume corrected for participant size (PCSAN). PCSAN was quantified using the following formula:

$$PCSAN = [(MV\ (m^3) / (BM\ (kg) \times Ht\ (m)) \times \cos(\theta)] / Lf\ (cm)$$

where BM is body mass and Ht is height of the participant.

Biomechanical Testing

During each test session, participants had three-dimensional (3D) lower limb (hip, knee, and ankle) joint biomechanics data recorded during a series of sports-relevant tasks. During each task, ground reaction force (GRF) data (2400 Hz) was recorded with a force platform (AMTI OR6 Series, Advanced Mechanical Technology Inc., Watertown, MA), while eight high-speed (240 Hz) optical cameras (MXF20, Vicon Motion Systems LTD, Oxford, UK) recorded 3D marker trajectories. For each trial, Vicon Nexus (v2.6, Vicon Motion Systems, LTD, Oxford, UK) captured and stored biomechanics data for post processing.

Each participant completed a variety of sports-relevant tasks. The tasks included an over-ground run, single leg cut, maximal vertical jump, drop landing, single-leg balance, and a sudden inversion event. The order of all tasks was randomized during the study for each testing session using a 6x6 Latin Square design (Table 3.3). For the purposes of this study, only the sudden inversion event task was analyzed. Data for the drop landing, vertical jump, and single-leg cut task are provided in Appendices C-E.

Table 3.3 The Latin Square design used for randomization of the activity order for each testing session

	Activity 1	Activity 2	Activity 3	Activity 4	Activity 5	Activity 6
Order 1	Balance	Cut	Run	Inversion	Land	Jump
Order 2	Cut	Inversion	Balance	Jump	Run	Land
Order 3	Inversion	Jump	Cut	Land	Balance	Run
Order 4	Jump	Land	Inversion	Run	Cut	Balance
Order 5	Land	Run	Jump	Balance	Inversion	Cut
Order 6	Run	Balance	Land	Cut	Jump	Inversion

For the sudden inversion event, participants stood with feet shoulder width apart, arms to the side, and looking straight ahead on a wooden platform. The wooden platform contained side-by-side trap doors that rotated 30 degrees when released, allowing the ankle to invert from a neutral standing position (Figure 3.2)[14,84]. Randomly, a researcher removed the mechanical support of one trap door, allowing the door to fall producing a sudden ankle inversion. Adhesive, non-slip strips on each trap door marked appropriate foot placement and prevent the foot from slipping when the trap door falls. The participant performed five successful trials on each leg. A trial was considered successful if the participant did not anticipate the inversion drop and stayed on the wooden platform for the duration of the trial. The order (dominant vs non-dominant) of the sudden inversion event trials was randomized with a random number generator (Excel 2016, Microsoft, Seattle, WA, USA) prior to testing.

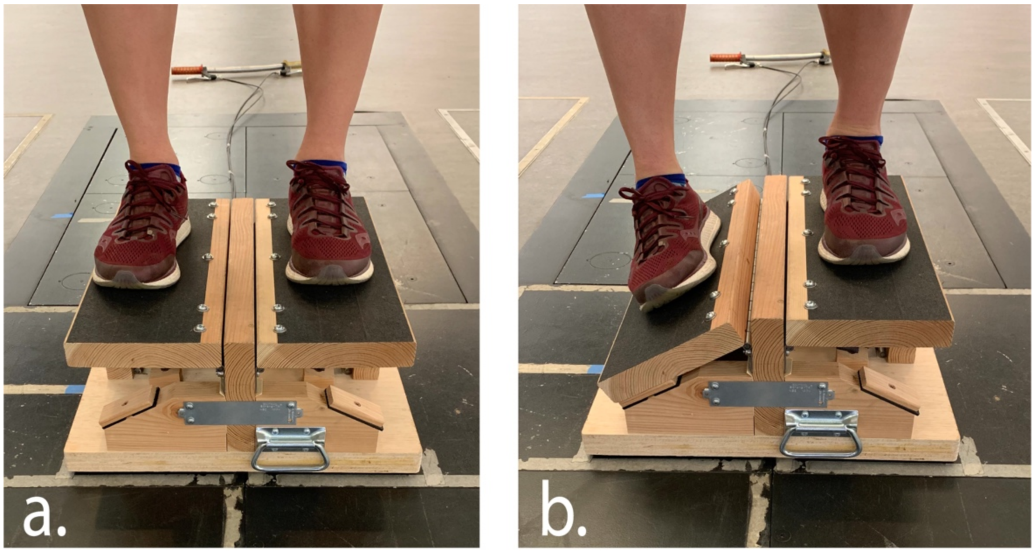

Figure 3.2 Depiction of (a) participant starting positon shoulder width apart on sudden inversion event platform, and (b) after the trap door is dropped, causing 30º of ankle inversion.

<u>Biomechanical Analysis</u>

During each trial, lower limb joint rotations were quantified from 3D coordinates of thirty-two retro-reflective markers (Table 3.4). Markers were applied to specific anatomical landmarks with double sided tape and secured with elastic tape (Cover-Roll Stretch, BSN Medical GmbH, Hamburg, Germany). After marker placement, a high-speed video recording of the participant standing in anatomical position (static) was recorded. The static recording was used to construct a kinematic model that consists of seven segments (pelvis, and bilateral thigh, shank, and foot) and 24 degrees of freedom. Each segment had a local coordinate system and three orthogonal axes (x, y, and z) assigned in Visual 3D. The orthogonal axes were specified using a joint coordinate system approach relative to the participants' static position that consists of medial-lateral x-axis, anterior-posterior y-axis, and vertical z-axis[90,91]. For the pelvis, the joint center was defined as halfway between the right and left anterior superior iliac spines (ASIS) and assigned six degrees of freedom (three rotational and three translational)[92]. For the hip, a functional joint center was calculated according to Schwartz and Rozumalski [93] and assigned a local coordinate system with three degrees of freedom. For the knee and ankle, the joint centers were calculated as the midpoint between the lateral and medial epicondyles and malleoli, respectively, and assigned three degrees of freedom according to Grood and Suntay[90], and Wu[36].

Table 3.4 Marker placement for kinematic model.

Segment	Marker Location
Pelvis	*Anterior-superior iliac spines, Posterior-superior iliac spines*, and Iliac crests
Thigh	Grater trochanter, Distal thigh, *Medial* and *lateral femoral epicondyles*
Shank	Tibial tuberosity, Lateral fibula, Distal tibia, *Medial* and *Lateral Malleoli*
Foot	Posterior heal, Midpoint of first and fifth metatarsal heads, *First metatarsal head*, and *Fifth metatarsal head*

Note: *Italic* indicates calibration markers

For the sudden inversion event, marker data was processed and low pass filtered using a fourth-order Butterworth filter cut off at 12 Hz[94]. The filtered marker trajectories were processed with Visual 3D (C-Motion, Rockville, MD) to solve for 3D hip, knee, and ankle rotations. All biomechanical variables during the sudden inversion event were time-normalized from 0-100% of inversion phase resampled to 1% increments (N=101). For the sudden inversion event, inversion phase was defined as the start of inversion movement to peak inversion plus 20 frames. The start of inversion was defined as the first instance ankle inversion exceeds two standard deviations above baseline (i.e., anatomical position), while peak inversion was defined by the largest ankle inversion angle exhibited during the sudden inversion event.

Statistical Analysis

For statistical analysis, specific ankle kinematic variables, and peroneal muscle parameters were submitted to analysis. For the sudden inversion event, the dependent variables include peak (0% - 100%) ankle plantarflexion and inversion angle, range (ROM; peak angle – initial angle) of plantarflexion and inversion motion, and time to peak ankle inversion. Each biomechanical variable was averaged across all successful trials to create a participant-based mean. Then, the participant-based mean was submitted to a repeated measures ANOVA to test the main effect and interaction of brace (ARG, Brace, Control, Tape) and sex (male vs. female). Significant interactions were submitted to simple effects analysis, and a Hommel Bonferroni correction was used for pairwise comparisons[95]. The peroneal muscle parameters submitted to analysis included PCSA, PCSAN and muscle stiffness, and maximum ankle plantarflexion, dorsiflexion, inversion, and eversion strength. Each dependent muscle parameter was submitted to an independent samples *t-test* to test the effect of sex. Intra- and inter-session reliability of peroneal muscle stiffness measurements was analyzed by the interclass correlation coefficient (ICC), and interpreted as follows: below 0.499 as poor, 0.500 to 0.699 as moderate, 0.700 to 0.899 as good, and 0.900 to 1.000 as excellent[96] (Appendix F.2). All statistical analysis was conducted with SPSS (v23, IBM, Armonk, NY) and alpha set to *a priori* at $P < 0.05$.

Results

During the sudden inversion event, a significant prophylactic by sex interaction was observed for ankle inversion ROM (p = 0.010). Females exhibited greater ankle inversion ROM compared to males with Control and ARG (p = 0.001, p = 0.010, adjusted α = 0.0125), but no significant difference was evident between sexes for any other condition (p > 0.017; Table 5). Females exhibited greater ankle inversion ROM with ARG compared to Brace (p = 0.001, adjusted α = 0.0125), and Control compared to Brace and Tape (p < 0.001, p < 0.001, adjusted α = 0.0125); whereas, males exhibited no difference in inversion ROM between any condition (p > 0.037; Table 3.5).

Table 3.5 Mean (SD) ankle biomechanics quantified during the sudden inversion event with each prophylactic for both sexes.

	ARG		Brace		Control		Tape	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
Pk Inv	33.78 (3.66)	36.58 (4.90)	33.73 (4.94)	35.18 (4.55)	32.68 (4.97)	38.32 (4.40)	31.72 (5.37)	36.02 (4.31)
Inv ROM	24.44 (2.68)	27.25 (3.12)	23.75 (3.41)	24.89 (3.87)	23.77 (4.22)	28.71 (2.78)	22.42 (4.49)	25.41 (4.16)
Dur	0.18 (0.05)	0.18 (0.04)	0.25 (0.06)	0.23 (0.06)	0.19 (0.05)	0.20 (0.04)	0.23 (0.05)	0.22 (0.04)
Pk PF	37.35 (5.13)	34.28 (4.33)	33.79 (5.38)	29.46 (4.94)	36.55 (5.09)	34.05 (3.52)	35.24 (4.62)	30.86 (5.60)
PF ROM	20.92 (3.39)	18.92 (3.85)	17.34 (3.81)	14.33 (3.94)	20.86 (3.35)	18.69 (3.85)	18.46 (3.62)	14.63 (3.83)

During the sudden inversion event, ankle prophylactic had significant effect on ankle inversion ROM (p < 0.001), time to peak inversion (p < 0.001), peak plantarflexion angle (p < 0.001), and plantarflexion ROM (p < 0.001), but not peak ankle inversion angle (p = 0.222). Ankle inversion ROM was greater with ARG and Control compared to Brace (p = 0.002, p = 0.002, p = 0.015, adjusted α = 0.0167) and Tape (p = 0.006, p < 0.001, adjusted α = 0.0167), but no significant difference was noted between any other condition ($p > 0.388$; Figure 3.3). During the sudden inversion event, it took significantly less time to reach peak inversion with ARG and Control compared to Brace ($p < 0.001$, $p < 0.001$, adjusted $\alpha = 0.0167$) and Tape ($p < 0.001$, $p = 0.001$, adjusted $\alpha = 0.0167$).

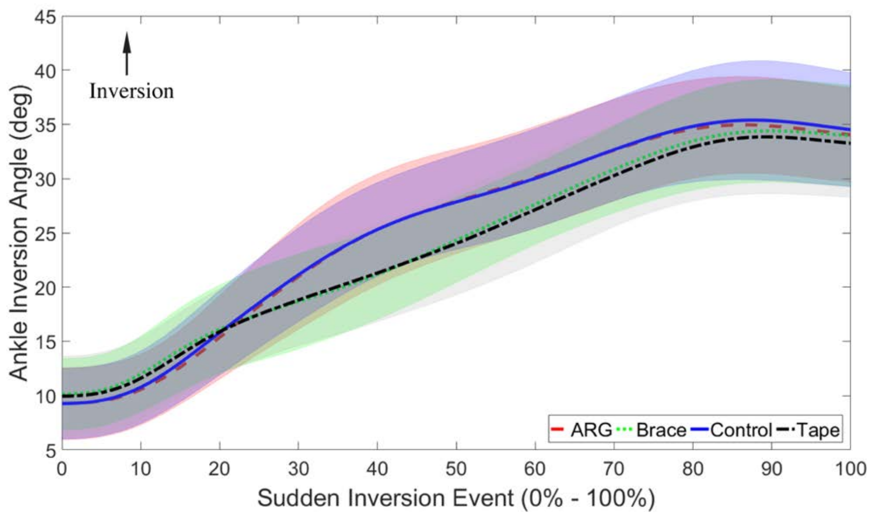

Figure 3.3 Mean (SD) ankle inversion angle for each prophylactic condition (ARG, Brace, Control, Tape) during the sudden inversion event (0-100%).

However, no difference in time to peak inversion was observed between ARG and Control ($p = 0.075$) or Brace and Tape ($p = 0.217$) in time to peak inversion (Table 5). Participants also exhibited greater peak ankle plantarflexion angles with Control ($p < 0.001$, adjusted $\alpha = 0.0125$) and ARG ($p < 0.001$, adjusted $\alpha = 0.0125$) compared to Brace and ARG compared to Tape ($p = 0.011$, adjusted $\alpha = 0.0125$), but no significant difference evident between any other condition ($p > 0.020$) (Figure 3.4). During the sudden inversion event, participants also exhibited significantly greater ankle plantarflexion range of motion with Control and ARG compared to Brace ($p < 0.001$, $p < 0.001$, adjusted $\alpha = 0.0167$) and Tape ($p < 0.001$, $p < 0.001$, adjusted $\alpha = 0.0167$). But, no difference between Control and ARG (p = 0.804) or Brace and Tape (p = 0.161) was evident.

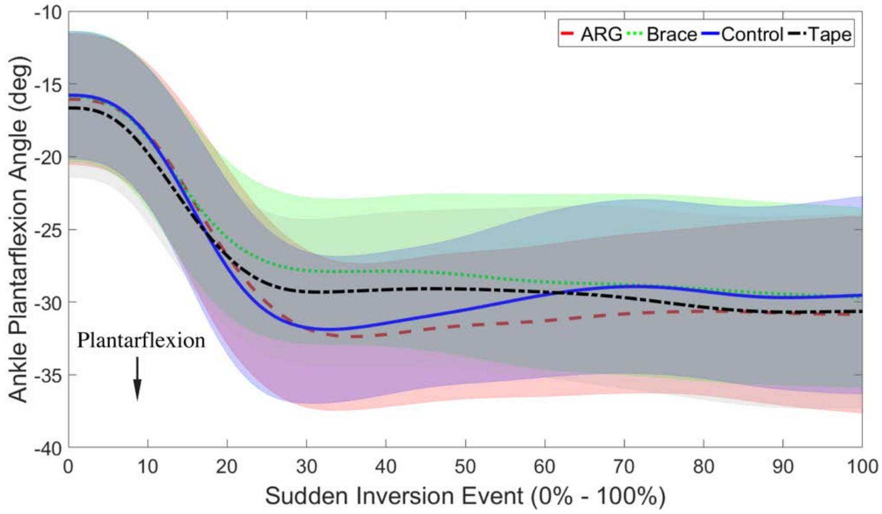

Figure 3.4 Mean (SD) ankle plantarflexion angle for each prophylactic condition (ARG, Brace, Control, Tape) during the sudden inversion event (0-100%).

Females exhibited greater peak ankle inversion angle (p = 0.009) and ROM (p = 0.011), and smaller peak plantarflexion angle (p = 0.012) and ROM (p = 0.021) compared to males (Figure 3.5, 3.6). Sex had no significant effect on time to peak inversion (p = 0.817).

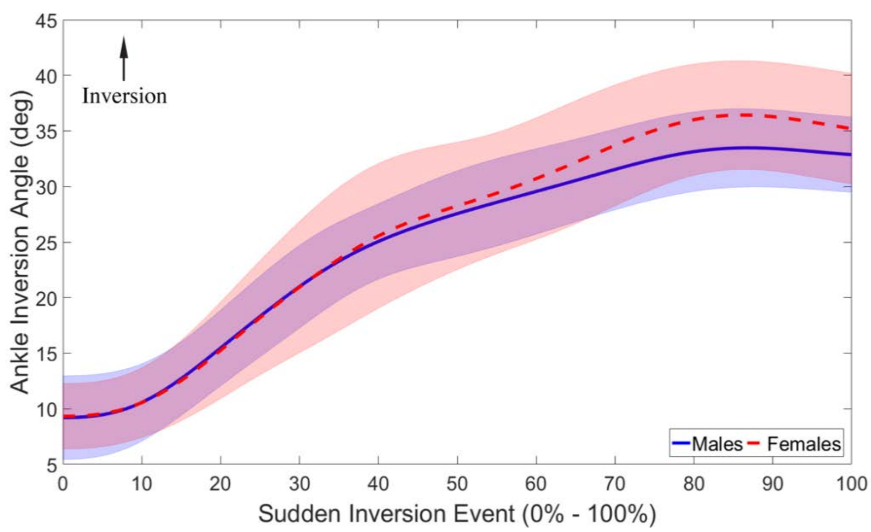

Figure 3.5 Mean (SD) ankle inversion angle between males and females for the sudden inversion event (0-100%).

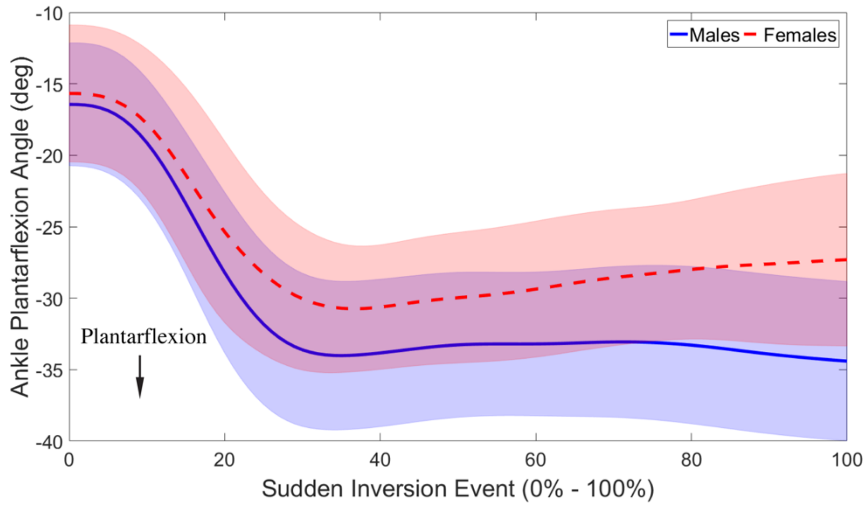

Figure 3.6 Mean (SD) ankle plantarflexion angle between males and females for the sudden inversion event (0-100%).

Females exhibited smaller PCSA ($p = 0.006$) compared to males. But after normalization for body mass, sex did not have a significant effect on peroneal size (PCSAN; $p = 0.735$) (Table 3.6). Further, females exhibited significantly smaller dorsiflexion strength ($p = 0.047$) compared to males. However, sex had no significant effect on or plantarflexion ($p = 0.106$), inversion ($p = 0.101$), or eversion ($p =0.142$) strength (Figure 3.7) or muscle stiffness (prone: $p = 0.196$, standing: $p = 0.488$, or inverted: $p = 0.804$) (Figure 3.8, Table 3.6).

Table 3.6 Mean (SD) muscle parameters for both sexes.

	PCSA	PCSAN	Prone	Standing	Inverted
Male	35.52 (10.16)	0.24 (0.08)	3.39 (0.91)	3.67 (1.34)	6.59 (1.40)
Female	25.29 (7.02)	0.23 (0.07)	2.94 (1.02)	3.37 (0.99)	6.73 (1.62)

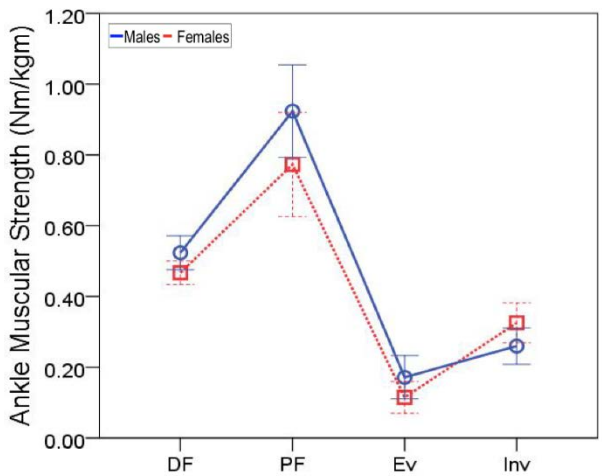

Figure 3.7 Mean (SD) dorsiflexion (DF), plantarflexion (PF), eversion (Ev), and inversion (Inv) muscular strength for males (blue) and females (red).

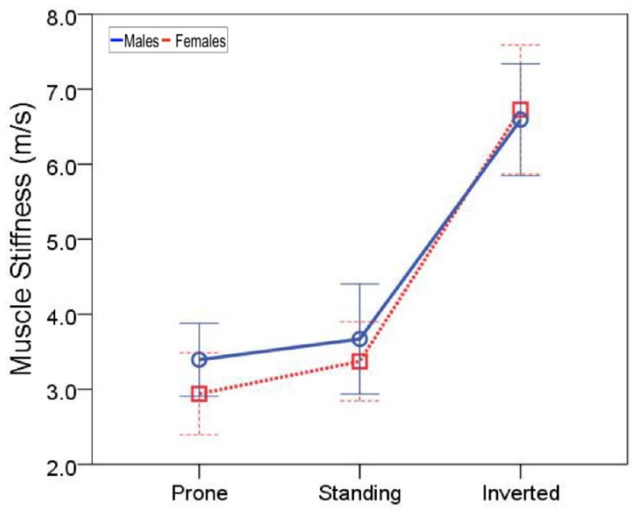

Figure 3.8 Mean (SD) peroneal muscle stiffness for males (blue) and females (red).

Discussion

Ankle prophylactics are used to prevent excessive joint inversion and subsequent injury, but the current outcomes support the tenet that prophylactic effectiveness to prevent injury may depend on device design[97]. In agreement with previous literature[8,9,62], both Brace and Tape may decrease risk of ankle sprain[8] by restricting ankle inversion

ROM (~1.9° to 2.3°) compared to an unbraced, Control ankle during the sudden inversion event. However, contrary to our hypothesis, the ARG may not effectively reduce ankle inversion ROM, as participants exhibited ~1.5° to 1.9° greater inversion ROM with ARG compared to Brace and Tape, respectively. While the reason for this discrepancy is not immediately evident, the ARG's design may only afford it the ability to limit harmful ankle motions, such as peak ankle inversion. In contrast with previous literature[45,46], peak ankle inversion angle exhibited during the sudden inversion event did not significantly differ between each ankle prophylactic. Participants did, however, exhibit a non-significant ~0.5° to 1.7° decrease in peak inversion with each brace (ARG, Brace, and Tape), compared to Control. Because ankle prophylactics reportedly reduce the incidence of ankle sprain[11,42], this small, statistically non-significant decrease in peak ankle inversion may hold clinical significance and warrants further study. Each ankle prophylactic (ARG, Brace and Tape), in fact, prevented peak ankle inversion angle from exceeding 36°, which is 5° below where injury is thought to occur[23]. Peak ankle inversion exhibited during the Control condition was also 4° lower than previously reported[98], and the current discrepancies with existing literature may stem from limitations of the wooden platform currently used for testing. The wooden platform included mechanical stops to prevent the participant from suffering an ankle sprain, but may have limited excessive ankle inversion and obfuscated the protective benefits of each ankle prophylactic.

An ideal prophylactic design would not restrict “normal" ankle motions, such as plantar-dorsiflexion, rather only prevent joint motions that lead to ligament damage and subsequent injury[99]. In agreement with McCaw & Cerullo[50], both Brace and Tape reduced ankle plantar- and dorsi-flexion motions with use. Specifically, Brace and Tape participants decreased peak plantarflexion angle between 2.2° and 3.7° compared to Control during the sudden inversion event. Ankle prophylactics that limit sagittal plane ankle motions may impair joint performance and increase the likelihood of mechanical or functional instability development[13,100]. The ARG produced a similar (i.e., not statistically significant) peak plantarflexion angle (35.8°) as the Control condition (35.3°), and subsequently may allow the user "normal" ankle motions, reducing the likelihood of mechanical or functional ankle instability development with use. Conversely, the current experimental outcomes demonstrate the ARG may be limited in its ability to stabilize the ankle compared to more restrictive braces, such as Brace and Tape. In agreement with previous literature[9,47] both Brace and Tape increased the time to peak inversion compared to Control by 18% and 15%, and compared to ARG by 24% and 23%, respectively. An increase in the time to peak inversion with the Brace and Tape prophylactics may allow the individual to coordinate a sufficient reflex response, potentially preventing ankle sprain[9], but may be indicative of the joint restriction that leads to functional joint instabilities and reduced user performance. Therefore, individuals who have suffered an ankle sprain may benefit from the use of ARG during rehabilitation, which allows the user "normal" ankle motions while providing restraint from harmful ankle motions (i.e., excessive inversion) that lead to re-injury.

The current outcomes indicate females' incidence of ankle sprain may stem from a sex dimorphism in ankle biomechanics. Females exhibited ~3.6° greater peak inversion angle and ~2.9° more ankle inversion ROM compared to males during the sudden inversion. Greater peak ankle inversion and inversion ROM are related to injury risk[8,24] and may predispose females to ankle injury[23,98], contributing to the sex disparity in injury rates. Yet, sex may also impact the effectiveness of an ankle prophylactic. Females reduced ankle inversion ROM with Brace (24.9°) and Tape (25.4°) compared to Control (28.7°), whereas, males exhibited no reduction in ankle inversion ROM with the ankle prophylactics. It may be restrictive prophylactics, such as Brace and Tape, only provide substantial protective benefits and effectively limit ankle inversion for increased ROMs exhibited by female participants.

During the sudden inversion event, females also displayed a sex dimorphism in sagittal plane ankle biomechanics. Contrary to our hypothesis, females decreased peak plantarflexion angle by 3.5º and ROM by 2.8° compared to males. While the reason for the contradiction with our hypothesis is not immediately evident, sagittal plane ankle motion is reported to reduce risk of ankle ligament damage[101], and may be related to function of ankle musculature[102]. Males exhibited 12% greater dorsiflexion strength and, albeit non-significant, 50% greater peroneal and 16% greater plantarflexion strength than females. Strength of males' ankle musculature may have afforded them the use of greater sagittal ankle plane motion to decrease risk of ankle sprain during the sudden inversion event. Conversely, the altered function of the ankle musculature for the typically smaller, weaker females may necessitate restricted sagittal plane ankle motions to limit the likelihood of injury. Females exhibited a significant 36% decrease in peroneal PCSA and non-significant 5% decrease in peroneal PCSAN compared to males. To combat their limited peroneal function, including size and strength, females may increase peroneal muscle activation of the ankle musculature[26] to provide the joint stability necessary to prevent injury, which manifests as reduced sagittal plane ankle motions during the sudden inversion event.

Muscle stiffness plays an important role in performance and injury. Increased levels of muscle stiffness leads to bony injuries, while decreased stiffness leads to soft tissue injuries[31]. Yet, to our knowledge, no study has reported differences in *in vivo* peroneal musculature stiffness between males and females. In contradiction of our hypothesis, sex had no significant effect on any peroneal muscle stiffness measurements (prone, standing, and inverted). However, males displayed a non-significant 17% and 9% increase in muscle stiffness in prone and standing positions, respectively, while females exhibited a 2% increase in muscle stiffness with an inverted ankle. Considering Akiyama et al.[32] reported greater muscle stiffness in individuals with current symptoms of medial tibial stress syndrome, it may be participants exhibiting symptoms of FAI, rather than sex, that presents altered peroneal muscle stiffness. In fact, participants who self-reported FAI symptoms exhibited up to a 13% increase in peroneal muscle stiffness compared to healthy controls (Appendix F.1). Increased stiffness measurements in FAI participants may indicate altered neuromuscular activation, and future is warranted to observe the peroneal muscle in FAI participants and muscle stiffness implications on ankle injury risk.

Conclusion

In conclusion, the use of ankle prophylactics may decrease risk of injury, but the protective benefits may depend on the specific prophylactic. During the sudden inversion event, all tested ankle prophylactics exhibited similar protection from excessive peak ankle inversion, yet participants only decreased ankle inversion ROM and time to peak inversion, biomechanics commonly associated with injury risk, with the Brace and Tape prophylactics. The restrictive Brace and Tape may also limit ankle sagittal plane motions, including peak plantarflexion angle and ROM, compared to the ARG, increasing the likelihood of mechanical or functional instability development with sustained use. During the sudden inversion event, females exhibited greater peak ankle inversion and ROM, biomechanics that may contribute to the sex disparity in ankle sprain. But, the tested prophylactics, in particular Brace and Tape, may only provide substantial protective benefits for females and not males. Further, the smaller and weaker peroneal females reduced sagittal plane ankle motions compared to males and future study is warranted to determine whether this stems from a sex dimorphism in peroneal size and strength.

CHAPTER FOUR: CONCLUSION

Introduction

This study's purpose was two-fold, (1) to examine the influence of ankle prophylactics on joint biomechanics during the sudden inversion event for both males and females, and (2) to determine if *in vivo* peroneal muscle parameters differ between sexes. Key findings support the hypotheses that ankle prophylactics reduce harmful biomechanics and subsequent joint injury risk, and females exhibit altered ankle biomechanics and peroneal function compared to males, which may increase their risk of ankle sprain.

Key Findings

Ankle prophylactics produced a significant reduction of frontal plane motion at the joint during the sudden inversion event, but this reduction differed between ankle prophylactics. Specifically, a greater reduction in ankle inversion and subsequent injury risk was exhibited with both Brace and Tape compared to the ARG and Control prophylactics. The Brace and Tape, also, limited sagittal plane ankle motions, increasing the likelihood of mechanical or functional instability development with sustained use. A sex dimorphism in ankle biomechanics was evident during the sudden inversion event. Females exhibited greater frontal plane ankle motion, which may contribute to the sex disparity in ankle sprain. Yet, the ankle prophylactics may be more effective for females, as they exhibited a greater reduction in ankle inversion compared to males with prophylactic use.

A sex dimorphism in peroneal muscle parameters was also evident. Females displayed smaller peroneal PCSA compared to males, however, after normalization to participant body mass, there was not a sex dimorphism in peroneal size. Females exhibited a reduction ankle muscle strength. But, despite being generally smaller and weaker, females did not exhibit a difference in peroneal muscle stiffness compared to males.

Significance

These findings support the tenet that the use of an ankle prophylactic can significantly decrease the risk of ankle sprain, but prophylactic effectiveness may depend on specific prophylactic and sex of user. This research provided the first documented sex-dimorphism in ankle biomechanics with prophylactic use. Females' ankle biomechanics, even with the aid of an ankle prophylactic, differ than males and may their increase risk of ankle injury. These experimental outcomes provided herein can be implemented by health care practitioners to improve treatment and rehabilitation strategies for initial ankle sprain. The implementation of these findings may lead to a reduction in functional ankle instability development. Further, the current findings provide fundamental knowledge regarding the etiology of ankle sprain in general and the ankle biomechanics specifically that produce the sex disparity in ankle injury rate.

Limitations

This study may be limited by the chosen ankle prophylactics. The currently chosen ankle prophylactics are commonly used during recreational activities and previous research. Yet, prophylactic effectiveness is dependent on specific device design and further testing of other ankle prophylactic designs may alter the current findings. However considering the general design is consistent across most ankle prophylactics, we do not anticipate testing different ankle prophylactics would have altered the practical significance of the current findings or resulted in significant different in peak ankle inversion angles exhibited during the sudden inversion event. The design of the wooden platform may be a limitation. The construction of the current wooden platform used during the sudden inversion event included mechanical stops that prevented an individual's ankle inversion from substantially exceeding 30°, and as a result we had no adverse events during testing. Although, these mechanical stops may have obfuscated the "true" peak ankle inversion angle, it was necessary for the health and safety of our participants. In particular, the design of the wooden platform may have limited the ability to quantify the ARG's effectiveness from its unique design. But, regardless, the wooden platform remains the gold standard for quantifying effectiveness of ankle prophylactics and allows from direct comparison with previously experimental data.

Future Work

The effectiveness of an ankle prophylactics may differ between male and female users. As such, future research is warranted to determine if ankle prophylactics need to be tailored to the specific sex of the user. A more restrictive prophylactic may be necessary for females to reduce the ankle inversion motions though to contribute to sex disparity in injury rate.

Females, who are generally smaller and weaker, may need tailored training or rehabilitation protocols to improve the peroneal function necessary to reduce initial or re-injury. Further, participants exhibiting current symptoms of FAI may exhibit increased peroneal muscle stiffness, indicating possibly altered neuromuscular activation. Future work should examine the peroneal muscle in individuals exhibiting current symptoms of FAI and implications of muscle stiffness of ankle injury risk.

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APPENDIX A

Ankle Instability Instrument

1. Have you ever sprained an ankle?

   Yes **No**

   If yes, which limb: **Rt** **Lt** **Both**

2. Have you ever seen a doctor for an ankle sprain?

   Yes **No**

3. Did you ever use a device (such as crutches) because you could not bear weight due to an ankle sprain?

   Yes **No**

4. Have you ever experience a sensation of your ankle "giving way"?

   Yes **No**

5. Does your ankle ever feel unstable walking on flat surface?

   Yes **No**

6. Does your ankle ever feel unstable walking on uneven ground?

   Yes **No**

7. Does your ankle ever feel unstable during recreational or sport activity?

   Yes **No**

8. Does your ankle ever feel unstable while going up stairs?

   Yes **No**

9. Does your ankle ever feel unstable while going down stairs?

   Yes **No**

APPENDIX B

TEGNER ACTIVITY LEVEL SCALE

Please circle that level that best describe your curve activity level.

Level 10	Competitive sports- soccer, football, rugby (national elite)
Level 9	Competitive sports- soccer, football, rugby (lower divisions), ice hockey, wrestling, gymnastics, basketball
Level 8	Competitive sports- racquetball or bandy, squash or badminton, track and field athletics (jumping, etc.), down-hill skiing
Level 7	Competitive sports- tennis, running, motorcars speedway, handball Recreational sports- soccer, football, rugby, bandy, ice hockey, basketball, squash, racquetball, running
Level 6	Recreational sports- tennis and badminton, handball, racquetball, down- hill skiing, jogging at least 5 times per week
Level 5	Work- heavy labor (construction, etc.) Competitive sports- cycling, cross-country skiing, Recreational sports- jogging on uneven ground at least twice weekly
Level 4	Work- moderately heavy labor (e.g. truck driving, etc.)
Level 3	Work- light labor (nursing, etc.)
Level 2	Work- light labor Walking on uneven ground possible, but impossible to back pack or hike
Level 1	Work- sedentary (secretarial, etc.)
Level 0	Sick leave or disability pension because of knee problems

APPENDIX C

Single-Leg Cut

For the single-leg cut, participants ran at 4 m/s ± 5% through the motion capture volume, planted their dominant foot on the designated force platform, and cut at 45 degrees towards the opposite side. Participants completed five successful cut trials. A successful trial consisted of the participants only contacting the force platform with the proper foot and running the correct speed measured with two sets of infrared timing gates.

For analysis, peak of stance (0%-100%) of ankle inversion joint angle and moment were quantified during the cut. Participant based means for each variable were submitted to a repeated measures ANOVA to test the main effect and interaction of brace (ARG, Brace, Control, and Tape) and sex (Male vs. Female). Significant interactions were submitted to simple effects analysis, and a Hommel Bonferroni correction used for pairwise comparisons.

Results

During the single-leg cut, prophylactic had no significant effect on peak ankle inversion angle ($p = 0.072$) or moment ($p = 0.059$; Table C.1). Females displayed significantly smaller peak inversion angle ($p = 0.027$) than males during the single-leg cut, but sex had no significant effect on peak ankle inversion moment ($p = 0.185$; Table C.1).

Table C.1 Mean (SD) ankle biomechanics for both sexes during the single-leg cut for both sexes with each prophylactic

	ARG		Brace		Control		Tape	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
AAA[a]	19.99 (6.81)	16.66 (6.28)	18.81 (5.95)	13.44 (5.07)	18.48 (7.61)	14.58 (4.88)	18.72 (6.11)	13.73 (4.98)
AAM (-)	0.19 (0.09)	0.13 (0.07)	0.17 (0.08)	0.13 (0.07)	0.16 (0.10)	0.11 (0.06)	0.16 (0.08)	0.15 (0.08)

[a] Denotes a significant main effect of sex.

APPENDIX D

Countermovement Jump

For the maximum countermovement jump, participants started in an athletic position with feet shoulder width apart on force platforms, before bending into a squat position and immediately performing a maximal effort vertical jump. Participants completed three successful jump trials. A successful trial consisted of the participant giving maximal effort as well as taking off and landing within the designated force platforms.

For analysis, maximal vertical jump height (m), calculate from time in air method according to Moir[103] and positive ankle joint work were quantified. Then a participant-based mean was submitted to a repeated measures ANOVA to test the main effect and interaction of brace (ARG, Brace, Control, and Tape) and sex (Male vs. Female). Significant interactions were submitted to simple effects analysis, and a Hommel Bonferroni correction used for pairwise comparisons.

Results

Prophylactic had a significant effect on jump height ($p = 0.007$) and positive ankle joint work ($p < 0.001$) during the countermovement jump task. With the tape prophylactic, participants maximal jump height was lower than with the ARG ($p = 0.004$, adjusted $\alpha = 0.0083$; Table D.1). However, no difference in jump height was evident between any other prophylactic ($p > 0.05$). Positive ankle work was smaller with both Brace and Tape compared to ARG ($p < 0.001$, $p < 0.001$, adjusted $\alpha = 0.0167$) and Control ($p < 0.001$, $p < 0.001$, adjusted $\alpha = 0.0167$; Table D.1), but there was no difference between Brace and Tape ($p = 0.454$), or ARG and Control ($p = 0.326$).

Females maximal jump height ($p < 0.001$) was lower and positive ankle joint work ($p < 0.001$) smaller than males during the countermovement jump task (Table D.1).

Table D.1 Mean (SD) jump height (JH) (m) and positive ankle work during the countermovement jump for both sexes with each prophylactic

	ARG		Brace		Control		Tape	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
JH[a,b]	0.42 (0.06)	0.26 (0.04)	0.41 (0.06)	0.25 (0.04)	0.42 (0.05)	0.25 (0.04)	0.41 (0.06)	0.25 (0.04)
AW[a,b]	74.09 (11.90)	54.45 (11.82)	64.49 (11.45)	48.88 (11.05)	75.24 (11.33)	56.09 (8.31)	69.06 (14.29)	46.82 (6.82)

a Denotes a significant main effect of sex.

b Denotes a significant main effect of brace.

APPENDIX E

Drop Landing

For the drop landing task, participants stepped off a 30 cm plyometric box and landed with each foot on a force platform. Each participant performed five successful trials. A successful trial consisted of the participant landing simultaneously on each foot that only contacts the respective force platform.

During the drop landing, peak vertical ground reaction force (vGRF) and ankle plantarflexion range of motion (ROM) were quantified for analysis. Participant-based means for each measure were submitted to a repeated measures ANOVA to test the main effect and interaction of brace (ARG, Brace, Control, and Tape) and sex (Male vs. Female). Significant interactions were submitted to simple effects analysis, and a Hommel Bonferroni correction used for pairwise comparisons.

Results

Prophylactic had a significant effect on ankle plantarflexion ROM ($p < 0.001$), but not peak vertical GRF ($p = 0.310$). With both Brace and Tape, participants exhibited less ankle plantarflexion ROM than compared to ARG ($p = 0.004$, $p = 0.004$, adjusted $\alpha = 0.0167$; Table E.1) and Control ($p < 0.001$, $p < 0.001$, adjusted $\alpha = 0.0167$; Appendix D.2). But, no difference was evident between Brace and Tape ($p = 0.475$), or ARG and Control ($p = 0.202$).

Females used greater ankle plantarflexion ROM ($p = 0.010$) than males. Sex had no effect on peak vertical GRF ($p = 0.858$; Table E.1).

Table E.1 Mean (SD) peak vertical GRF (BW) and plantarflexion ROM (°) during the drop landing for both sexes with each prophylactic

	ARG		**Brace**		**Control**		**Tape**	
	Male	**Female**	**Male**	**Female**	**Male**	**Female**	**Male**	**Female**
GRF	1.60	1.67	1.56	1.57	1.60	1.65	1.62	1.64
	(0.65)	(0.58)	(0.64)	(0.53)	(0.67)	(0.52)	(0.74)	(0.53)
ROM[a,b]	45.48	53.46	42.18	47.27	46.26	54.67	42.91	47.85
	(6.69)	(10.41)	(6.72)	(8.49)	(6.02)	(8.16)	(6.50)	(6.61)

a Denotes a significant main effect of sex.

b Denotes a significant main effect of brace.

APPENDIX F

Peroneal Muscle Stiffness for FAI Participants

The peroneal muscle stiffness values calculated for control and FAI participants are presented Table F.1. The FAI participants, who indicated a history of medically diagnosed ankle sprain accompanied by frequent sensations of “giving way”[15], exhibited a non-significant (between 5% to 13%) increase in prone ($p = 0.654$), standing ($p = 0.618$), and inverted ($p = 0.131$) muscle stiffness compared to Con participants.

Table F.1 Mean (SD) peroneal muscle stiffness (m/s).

	Prone	Standing	Inverted
Con	3.10 (1.12)	3.43 (1.25)	6.31 (1.64)
FAI	3.26 (0.79)	3.64 (1.14)	7.11 (1.18)

The reliability (both intra- and inter-session) recorded for peroneal muscle stiffness measurements are presented in Table F.2. Reliability was determined by calculating interclass correlation coefficient (ICC) from peroneal stiffness collected by one investigator (WDI) on four consecutive days. ICC values below 0.499 were interpreted as poor, 0.500 to 0.699 as moderate, 0.700 to 0.899 as good, and 0.900 to 1.000 as excellent[97]. The calculated intra-session reliability was determined to be moderate (ICC = 0.876), whereas, the inter-session measurements were determined to be good (ICC = 0.646), respectively.

Table F.2 Mean (SD) peroneal muscle stiffness (m/s) reliability measurements.

	Mean ± SD (m/s)	95% CI	ICC
Trial-to-Trial	5.141 ± 1.069	(0.277 - 0.997)	0.876
Day-to-Day	5.124 ± 1.010	(0.409 - 0.855)	0.646