THE EFFECTS OF FUNCTIONAL ANKLE INSTABILITY AND INDUCED FATIGUE ON ANKLE STIFFNESS.

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Abstract

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The Effects of Functional Ankle Instability and Induced Fatigue on Ankle Stiffness.
(Under the direction of Steven Zinder Ph.D, ATC)

**Objective:** To investigate effects of fatigue and functional ankle instability on ankle stiffness. **Design:** Repeated measures pre-fatigue, post-fatigue measurement of ankle stiffness in stable and functionally unstable ankles. **Subjects:** Forty subjects (14 men and 26 women, age = 21.7 ± 2.5 years, weight = 173.3 ± 11.0 kilograms, height = 74.7 ± 20.9 centimeters) assigned to stable ankle and functionally unstable ankle groups. **Statistical Analysis:** Three 2 (stable, functionally unstable) x 2 (pre-fatigue, post-fatigue) mixed model repeated measures ANOVAs. **Main Outcome Measure(s):** Ankle stiffness, peroneus longus EMG pre-activation, and peroneus longus mean EMG amplitude. **Results:** No significant differences in ankle stiffness and significant decreases in EMG pre-activation and mean EMG amplitude post-fatigue. **Significance:** Fatigue alters EMG pre-activation and EMG amplitude but the effect on ankle stiffness remain unclear due to lack of appropriate peroneus fatigue in this study. **Key Words:** Ankle stiffness, functional ankle instability, peroneus longus fatigue.
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Ankle injuries are common among the adult athletic population in the United States. Currently, ankle injuries account for five percent of all sports injuries and two billion dollars in medical costs annually (Birrer, Fani-Salek et al. 1999). Eighty-five percent of these injuries are classified as ankle sprains (Freeman, Dean et al. 1965). There is agreement throughout the current literature that an initial acute ankle sprain can be associated with the development of functional instability. Functional ankle instability is commonly described as self-reported instability, or the feeling of “giving way” that is present in up to 40 percent of patients with a history of lateral ankle sprain (Freeman, Dean et al. 1965; Tropp 1986). While this simple definition has been widely used, current literature has clearly shown that functional ankle instability is a complex multi-factorial condition that is not yet fully understood (Docherty, Arnold et al. 2004).

Currently, there are no widely accepted criteria for diagnosis of functional ankle instability. It has been proposed that functional ankle instability is a self reported sensation of repeated giving way with no regard for the severity of initial injury, instability following a severe ankle sprain, loss of kinesthesia and position sense following lateral ankle sprain, or some combination of factors that remains poorly understood (Tropp 1986; Konradsen 2002; Vaes, Duquet et al. 2002; Docherty, Arnold et al. 2004). Despite these various definitions, it has been shown that lateral ankle injuries initiate a sequence of physiological events that lead
to a decreased ability to respond to injurious perturbations during daily functional activities (Vaes, Duquet et al. 2002).

Several factors have been theorized to be responsible for the alteration in ankle stability following lateral ankle injury present in those with functional ankle instability. The most common factor in the literature is an alteration in afferent pathways that may effect reflex muscle activation in response to sudden ankle inversion. (Freeman, Dean et al. 1965; Konradsen and Ravn 1990; Fernandes, Allison et al. 2000; Vaes, Duquet et al. 2002; McVey, Palmieri et al. 2005). Investigations into reflex muscle activation have focused on the peroneus longus, peroneus brevis, and tibialis anterior muscles of healthy subjects and subjects with functional ankle instability and have shown inconsistent results (Vaes, Duquet et al. 2002). Some studies have demonstrated significant differences in muscle activation timing (Fernandes, Allison et al. 2000) and amplitude, while others report no significant results (Konradsen and Ravn 1990; Fernandes, Allison et al. 2000). Reflex muscle activation may play a role in ankle stability, however the ability to rapidly produce force that is equal to that of the perturbation has been argued to be the key to stability during functional activity rather than the measurement of EMG activity (Padua, Garcia et al. 2005). Therefore, it is essential to understand not only the reflex characteristics, but also the differences in force producing characteristics that are present in stable versus functionally unstable ankles.

Functional activity can be broadly defined as normal daily activity, weight bearing activity, or more specifically as normal bouts of weight bearing athletic activity depending on the setting and population in which the investigator is interested. The majority of literature regarding functional ankle instability has focused on recreationally active subjects at rest in a laboratory setting. While this research is essential in clarifying both the causes and effects of
ankle instability, it does not help to illuminate the functional aspect of this condition. Functional activity can lead to fatigue if it is of a sufficient volume, duration, or intensity. During repetitive daily activity and athletic participation, fatigue occurs at both the spinal and muscular levels (Edwards 1981; Kent-Braun 1999; Dimitrova and Dimitrov 2003; Enoka and Duchateau 2008; Hunter, Yoon et al. 2008). This fatigue leads to alteration in both muscle activation and force production that may affect the ability of dynamic restraints such as muscles to provide maximal stability to a joint. Currently, the effects of functional ankle instability during a fatiguing activity have not been studied in depth. However, these effects may be most important to both the recreationally active and athletic populations due to high occurrence of functional instability within those populations and its potential effects on injury rates and performance (Freeman, Dean et al. 1965; Birrer, Fani-Salek et al. 1999; Docherty, Arnold et al. 2004).

It has been hypothesized that an alteration in ankle stiffness due to fatigue may lead to increased predisposition to lateral ankle sprain during athletic activity (Johnston, Howard et al. 1998). Joint stability is essential to preventing ankle sprain due to excessive motion during activity and is affected by ankle joint stiffness, which is the amount of resistance to external force or perturbation. By definition, stiffness is the force response to perturbation divided by length change of passive and active structures surrounding a joint (Wagner and Blickhan 1999; Zinder, Granata et al. 2007). Therefore any lack in the ability to effectively produce force may decrease ankle stiffness and therefore decrease the stability of the ankle joint. At the ankle, stiffness is considered to be an effective measure of joint stability and acts as a clear measure of the inherent protective mechanisms to injurious perturbation at the ankle. Fatigue is defined as the decrease in ability to produce force and may be an important
factor when attempting to understand the contributions of intrinsic and extrinsic factors that lead to functional ankle instability (Edwards 1984; Vollestad 1997). It is currently unclear whether the decreased ability to produce force associated with muscle fatigue has a direct effect on stability; however, because of its potential effect on joint stiffness, it can be hypothesized that an alteration in ankle stiffness due to fatigue may lead to increased predisposition to lateral ankle sprain during athletic activity (Johnston, Howard et al. 1998). Therefore, the purpose of this study was to measure the effects of induced fatigue on muscle activation and ankle stiffness in response to frontal plane ankle oscillation in healthy subjects and subjects with functional ankle instability.

Independent Variables

- Ankle Stability Status (2 levels)
  - Stable ankle
  - Functionally unstable ankle
- Fatigue Status (2 levels)
  - Baseline
  - Post-fatigue

Dependent Variables

- Ankle stiffness
- Peroneus longus mean EMG pre-activation
- Peroneus longus mean EMG amplitude
**Research Questions**

- What is the effect of ankle stability on ankle stiffness?
- What is the effect of fatigue status on ankle stiffness?
- What are the interaction effects of ankle stability and fatigue status of ankle stiffness?
- What is the effect of ankle stability on peroneus longus mean EMG amplitude during frontal plane ankle oscillation?
- What is the effect of fatigue status on peroneus longus mean EMG amplitude during frontal plane ankle oscillation?
- What are the interaction effects of ankle stability and fatigue status of peroneus longus mean EMG amplitude?
- What is the effect of ankle stability on peroneus longus mean EMG pre-activation during frontal plane ankle oscillation?
- What is the effect of fatigue status on peroneus longus mean EMG pre-activation during frontal plane ankle oscillation?
- What are the interaction effects of ankle stability and fatigue status of peroneus longus mean EMG pre-activation?

**Research Hypotheses**

- It is hypothesized that there was no significant difference in ankle stiffness in functionally unstable ankles when compared to stable ankles.
- It is hypothesized that there was no significant difference in ankle stiffness at post-fatigue when compared to baseline.
• It is hypothesized that there was no interaction effect of ankle stability and fatigue status on ankle stiffness.

• It is hypothesized that there was a significant increase in peroneus longus mean EMG amplitude in functionally unstable ankles when compared to stable ankles.

• It is hypothesized that there was a significant increase in peroneus longus mean EMG amplitude post-fatigue when compared to baseline.

• It is hypothesized that there was an interaction effect of ankle stability and fatigue status on peroneus longus mean EMG amplitude.

• It is hypothesized that was significantly greater peroneus longus mean EMG pre-activation in functionally unstable ankles when compared to stable ankles.

• It is hypothesized that was significantly greater peroneus longus mean EMG pre-activation post-fatigue when compared to baseline.

• It is hypothesized that was an interaction effect of ankle stability and fatigue status on mean EMG pre-activation.

**Statistical Hypotheses**

• There was no significant difference in ankle stiffness in functionally unstable ankles when compared to stable ankles. The alternative hypothesis is that there was a significant difference in ankle stiffness in functionally unstable ankles when compared to stable ankles.

• There was no significant difference in ankle stiffness at baseline and post-fatigue. The alternative hypothesis is that there was a significant difference in ankle stiffness at baseline and post-fatigue.
• There was no significant interaction between ankle stability and time on ankle stiffness. The alternative hypothesis is that there was a significant interaction between ankle stability and time on ankle stiffness.

• There was a significant increase in mean peroneus longus EMG amplitude in functionally unstable ankles when compared to stable ankles. The alternative hypothesis is that there was no significant difference in mean peroneus longus EMG amplitude in functionally unstable ankles when compared to stable ankles.

• There was a significant increase in mean peroneus longus EMG amplitude at baseline and post-fatigue. The alternative hypothesis is that there was no significant difference in mean peroneus longus EMG amplitude at baseline and post-fatigue.

• There was a significant interaction between ankle stability and time on mean peroneus longus EMG amplitude. The alternative hypothesis is that there was no significant interaction between ankle stability and time on peroneus mean peroneus longus EMG amplitude.

• There was a significant increase in peroneus longus mean EMG pre-activation in functionally unstable ankles when compared to stable ankles. The alternative hypothesis is that there was no significant difference in peroneus longus mean EMG pre-activation in functionally unstable ankles when compared to stable ankles.

• There was a significant increase in peroneus longus mean EMG pre-activation at baseline and post-fatigue. The alternative hypothesis is that there was no significant difference in peroneus longus reflex amplitude at baseline and post-fatigue.
• There was a significant interaction between ankle stability and time on peroneus longus mean EMG pre-activation. The alternative hypothesis is that there was no significant interaction between ankle stability and time on peroneus longus reflex amplitude.

Operational Definitions

• Physically active: participation in varsity, club, intramural, or recreational athletic activity at least three days a week for no less then 30 minutes per session.

• Limb dominance: the limb with which a subject would strike a ball for maximum distance.

• Muscle fatigue: the inability to maintain expected muscular force or power output. Muscle fatigue will be measured the ability to sustain a 20% eversion maximum volitional isometric contraction without falling below 10% of the maximum volitional isometric contraction for five consecutive seconds (Hunter, Rochette et al. 2005).

• Functional ankle instability: the repeated sensation of giving way at the ankle in subjects with a history of sprain of the involved ankle that required non-weight bearing status or a visit to a physician for care. The ankle stability of subjects will be assessed via self-report on the Ankle Instability Instrument (AII) (Docherty, Arnold et al. 2004).

• Peroneus longus mean EMG amplitude: the mean EMG amplitude measured 500 milliseconds immediately following perturbation of the inversion-eversion swaying cradle device.

• Peroneus longus EMG pre-activation: the mean EMG activity 250 milliseconds prior to initiation of perturbation on the inversion-eversion swaying cradle device.
Assumptions

- Surface EMG is a reliable and valid measure of muscle activity in the peroneus longus muscle (Kollmitzer, Ebenbichler et al. 1999).
- The inversion-eversion swaying cradle device is a valid and reliable measure of inversion-eversion ankle stiffness (Zinder, Granata et al. 2007)
- The sustained hold isometric fatigue protocol will produce a true level of peripheral fatigue in the peroneus longus.

Delimitations

- Subjects were recruited from the population of the University of North Carolina.
- The sample was comprised of physically active adults between the ages of 18-30 years old.
- Functional ankle instability was assessed via self-report on the AII (Docherty, Arnold et al. 2004).
- Subjects received a pre-test, sustained hold isometric fatigue protocol, and post-test within the same testing session.
- Surface EMG measurements were limited to the peroneus longus muscle.
- Measurement of fatigue was limited to fatigue of the peroneus longus muscle.

Limitations

- Functional ankle instability was determined through self-reported responses to the AII.
- Ankle inversion-eversion oscillation was performed in a non-functional position.
• Ankle motion was limited to the frontal plane by the inversion-eversion swaying cradle device.

• Peripheral fatigue of peroneus longus was achieved via a non-functional eversion sustained hold isometric fatigue protocol at 20% of maximum volitional isometric contraction.
Chapter 2

Review of Literature

Introduction

The purpose of this review is to present current literature related to ankle stiffness in healthy and functionally unstable ankles following peripheral muscle fatigue. It is clear that functional ankle instability causes physiological and functional alterations at the ankle joint, however the details and scope of these changes are not yet entirely understood. In order to better understand the potential effects of fatigue on stiffness at the ankle joint, it is first important to clearly outline what is known about the causes and the potential physiological and functional effects of functional ankle instability in the active population. We will then present the various types of fatigue that occur in the body during functional and non-functional activities. Clarifying these types of fatigue will allow us to show why this study will focus on peripheral muscle fatigue in the hope of showing its potential effects on dynamic stiffness in functionally unstable ankles. We will then present what is currently known about ankle stiffness at rest and during functional activity in order to highlight its potential importance in stability and its possible link to functional ankle stability. While a tremendous amount of research is available in these areas, the interaction of functional ankle instability and fatigue on ankle stiffness has not been fully explored and may lead to greater clarity in regards to risk factors for ankle injury during athletic activity. Therefore our
research questions is: What are the effects of functional ankle instability and fatigue on ankle stiffness?

**Functional Ankle Instability**

Functional ankle instability (FAI) is a condition that has been shown to occur in up to 40 percent of patients who have experienced a lateral ankle sprain (Bosien, Staples et al. 1955; Freeman, Dean et al. 1965). Five to ten million ankle injuries, 85 percent of which are considered lateral ankle sprains, occur annually in the United States. It has been shown that adults between 21-30 years of age are at the greatest risk for lateral ankle injury with participation in athletic activity further increasing risk (Gerrick and Requa 1988). It has been shown that in 1991 roughly 23,000 ankle sprains occurred daily, which equals 1 injury per 10,000 people. Therefore, using current population estimates, it is likely that this number is now closer to 28,000 ankle sprains daily (Kannus and Renstrom 1991). In some athletic activities, it has been shown that following initial sprain, repetitive injury can be present in up to 70 percent of patients, and residual symptoms including pain and swelling may be present in 55-72 percent of patients for 6-18 months (Gerber, Williams et al. 1998; Braun 1999). The cause of functional ankle instability is multi-factorial and includes both anatomical and behavioral components; however, it is clearly linked to previous sprain of the lateral ankle (Bosien, Staples et al. 1955; Freeman, Dean et al. 1965; Garrick and Requa 1988; Kannus and Renstrom 1991; Yeung, Chan et al. 1994; Konradsen, Voigt et al. 1997; Gerber, Williams et al. 1998; Birrer, Fani-Salek et al. 1999; Braun 1999; Hertel 2000; McKay, Goldie et al. 2001; Beynon, Murphy et al. 2002). Due to increased exposures to athletic activity and therefore increased risk of sprain due to exposure, active adults may be
at greater risk of developing functional ankle instability when compared to less active populations (Freeman, Dean et al. 1965; Gerber, Williams et al. 1998; Braun 1999).

Functional ankle instability is a widely studied condition within sports medicine research, however, there is no widely accepted definition or inclusion criterion for this condition. Early definitions of functional ankle instability were simply a history of recurrent lateral ankle sprains and a self reported subjective feeling of giving way at the foot or ankle (Freeman, Dean et al. 1965). While this definition is lacking in diagnostic criteria and more detailed subjective complaints, it remains one of the most widely used definitions in the field. A more recent definition that has been used to define this condition is recurrent ankle instability and the sensation of joint instability due to the contributions of proprioceptive and neuromuscular deficits (Hertel 2000). This definition is not as widely utilized in current research, but it appears to be a more complete and clinically applicable definition that incorporates both clinical symptoms and anatomical causation of functional instability (Hertel 2000; Hertel 2002; Mora, Quinteiro-Blondin et al. 2003; Hertel 2005; McVey, Palmieri et al. 2005). These two aforementioned definitions are by far the most commonly used for describing functional ankle instability, however, there are a limited number of studies that have also included decreased eversion strength as a factor within their definition (Tropp 1986; Kaminski and Hartsell 2002; Hubbard, Kramer et al. 2007; Hubbard, Kramer et al. 2007). These definitions are widely utilized within functional ankle instability research, however, it can be seen in the lack of detail and overall disagreement within the literature that there is no one definitive definition for functional ankle instability and that the available definitions may not take into account the complex nature of the condition.
While many causes of functional ankle instability have been studied, it is unclear why this condition develops in such a high percentage of injured adults (Freeman, Dean et al. 1965; Hertel 2000; Hertel 2002; Hertel 2005; Hubbard and Hertel 2006; Hubbard, Kramer et al. 2007; Hubbard, Kramer et al. 2007). Authors have proposed several mechanisms which produce functional ankle instability, the most common of which include alterations in afferent nerve activity, delayed reflex activation, decreased strength of the foot evertors, and mechanical instability of the ankle, all of which occur following lateral ankle injury (Freeman, Dean et al. 1965; Tropp 1986; Konradsen and Ravn 1991; Ebig, Lephart et al. 1997; Fernandes, Allison et al. 2000; Kaminski and Hartsell 2002; Vaes, Duquet et al. 2002; Hertel 2005; McVey, Palmieri et al. 2005; Hubbard and Hertel 2006; Gribble, Hertel et al. 2007). While none of these factors have been shown to occur in all patients with functional ankle instability, de-afferentiation, or alterations in mechanoreceptor activity within the ankle joint and surrounding tissues, appears to be the most common factor represented in the literature (Freeman, Dean et al. 1965; Hertel 2002; Hertel 2005). The theory of de-afferentiation was originally proposed by Freeman, et al. in 1965, but has been continually studied as a potential cause of functional ankle instability following lateral ankle sprain. In the original description, it was proposed that because afferent fibers lie in ligaments and capsules which have lower tensile strength than other dynamic supports of the ankle joint, nervous tissue was very likely to be injured during a tensile injury (Freeman, Dean et al. 1965). This neurologic deficit then leads to a decrease in stabilization reflexes during locomotion due to decreased afferent nerve activity, which provides the person the subjective feeling of giving way (Freeman, Dean et al. 1965). H:M ratios are a comparison of the maximal number of alpha motor neurons that can be excited by the central nervous system.
compared with the maximal number of motor neurons available to be activated (McVey, Palmieri et al. 2005). This is a significant marker of arthrogenic inhibition and may be a link to the initial description of de-afferentiation seen in early functional ankle instability research (Freeman, Dean et al. 1965; McVey, Palmieri et al. 2005). Study of the H:M ratios in the soleus and peroneal muscles of functionally unstable ankles have shown a decreased H:M ratio when compared to healthy ankles. This shows a possible confirmation of de-afferentiation in functionally unstable ankles due to the fact that the decrease in H:M ration shows a inability to facilitate the transmission of normal afferent information to ankle musculature. Since its original description, research on de-afferentiation has taken very diverse paths including the study of peroneal reaction time and amplitude, and alterations in postural stability in patients with functional ankle instability.

Peroneal EMG latency and electromechanical delay are two of the largest areas of study in relation to dynamic stability in healthy and functionally unstable ankles. Ankle inversion is the primary cause of lateral ankle sprain and the main direction of instability in functionally unstable patients. Therefore, it is thought that an alteration in neural activation or a decreased ability to rapidly evert the foot in response to inversion perturbation by the peroneal muscles may lead to an increased feeling of instability when compared to healthy patients (Bosien, Staples et al. 1955; Freeman, Dean et al. 1965; Konradsen and Ravn 1990; Konradsen, Voigt et al. 1997; Hertel 2000; Beynon, Murphy et al. 2002; Vaes, Duquet et al. 2002; McVey, Palmieri et al. 2005). In healthy patients, the spinal stretch reflex leads to activation of the peroneus longus and peroneus brevis in response to inversion perturbations via a mono-synaptic afferent pathway (Konradsen and Ravn 1990; Konradsen, Voigt et al. 1997). While it is clear that a reflex mechanism is activated via sudden inversion of the
ankle, it is unclear how helpful this mechanism is in preventing lateral ankle injury due to sudden inversion. Several studies have attempted to establish the ability of the spinal stretch reflex to prevent injurious ankle inversion in healthy patients. In all cases, electromyographical data were recorded from the peroneal muscles of healthy adult subjects during a sudden inversion on a trapdoor platform. Konradsen, et al. completed a study with ten healthy subjects who had a mean age of 28 years. Patients were full weight bearing on a trapdoor platform and suddenly inverted to 30 degrees. This study demonstrated a median peroneal latency, or the time from trapdoor release to first significant EMG activation of the peroneal muscles to be 54ms and the electromechanical delay, or time from trapdoor release to first movement of the foot into eversion to be 126ms (Konradsen, Voigt et al. 1997). When compared to the estimated 100ms required to reach 40 degrees of inversion, a proposed injurious angulation in this experimental design, it becomes clear that peroneal reflex activation may not be timely enough to provide significant dynamic support to prevent sudden inversion injury (Konradsen, Voigt et al. 1997). These results have been confirmed by several other similar studies (Lynch, Eklund et al. 1996; Konradsen, Voigt et al. 1997; Benesch, Putz et al. 2000; Fernandes, Allison et al. 2000). While there is great variability in the methods of measurement of peroneal reaction time and EMG latency, results have consistently showed that peroneal reflexes in healthy ankles are not able to the required force to prevent sudden inversion injury (Lynch, Eklund et al. 1996; Konradsen, Voigt et al. 1997; Benesch, Putz et al. 2000; Fernandes, Allison et al. 2000; Konradsen, Peura et al. 2005). However, with the addition of peroneal muscle pre-activation, force production may occur in a much more timely manner in order to provide greater ankle stability (Konradsen, Peura et al. 2005). In a recent study, time to maximal eversion torque production in response to
sudden inversion perturbation was measured under relaxed and pre-activated conditions. This study showed a significant decrease in time to maximum eversion torque, from 326ms to 89ms, when subjects with relaxed peroneal muscles were compared to those with pre-activated peroneal muscles (Konradsen, Peura et al. 2005). While there is not currently enough in the literature to say that muscle pre-activation or preparatory EMG activity is a more important measure than muscle reflex EMG characteristics when attempting to understand the contribution of muscle activation to ankle stability, it is becoming clear that preparatory EMG activity may be an area of interest due to its apparent positive effect time to torque production following sudden inversion.

Peroneal EMG latency and electromechanical delay have been thoroughly studied in the functionally unstable ankle. It is clear in studies that have investigated both latency and electromechanical delay that in healthy ankles, spinal stretch reflexes may not be rapid enough to produce adequate force in response to sudden inversion. However, it is not clear whether there are significant differences in these variables between healthy and functionally unstable ankles (Konradsen and Ravn 1990; Konradsen and Ravn 1991; Beckman and Buchanan 1995; Ebig, Lephart et al. 1997; Fernandes, Allison et al. 2000; Vaes, Duquet et al. 2002). Several studies have found no statistically or clinically significant difference in peroneal EMG latency via inversion platform perturbations or electromechanical delay via a non-weight bearing load cell apparatus when comparing healthy and functionally unstable ankles (Beckman and Buchanan 1995; Ebig, Lephart et al. 1997; Fernandes, Allison et al. 2000; Vaes, Duquet et al. 2002). A study by Mora, et al. measured electromechanical delay during monopedal and bipedal stance via common peroneal nerve stimulation in college aged functionally unstable and healthy ankles. No significant difference was found in
electromechanical delay between groups, and an increase in force production was seen in functionally unstable ankles in monopedal stance when compared to bipedal stance (Mora, Quinteiro-Blondin et al. 2003). It has been hypothesized that this increase in force production is most likely due to the increased need for dynamic support when attempting to stabilize on a functionally unstable ankle due to an alteration in intrinsic ankle stabilizers (Mora, Quinteiro-Blondin et al. 2003). Only two studies found a statistically significant difference in peroneal EMG latency between functionally unstable and healthy ankles during a sudden inversion perturbation (Konradsen and Ravn 1990; Konradsen and Ravn 1991). However, neither study recorded electromechanical delay. Therefore it is not clear whether this difference in EMG latency can be considered clinically relevant due to a lack of data related to force output. While the peroneal muscles may have been active, it is unclear at what point force was produced and therefore impossible to tell whether the reflex response would have any clinical significance for injury prevention or differences in subjective feelings of instability. Peroneal muscle reflexes and activity are some of the most frequently researched topics in regard to functional ankle instability. Currently, there is no consensus as to the effects of functional ankle instability on peroneal activation however, the research tends to support the idea that peroneal reactions are not sufficient to prevent ankle inversion during sudden perturbation in both healthy and functionally unstable ankles (Beckman and Buchanan 1995; Ebig, Lephart et al. 1997; Fernandes, Allison et al. 2000; Vaes, Duquet et al. 2002). While there is some evidence supporting differences in EMG latency between functionally unstable and healthy ankles, it is unclear whether this difference has any clinical significance due to the lack of investigation of electromechanical delay in those studies in which a difference was found (Konradsen and Ravn 1990; Konradsen and Ravn 1991).
Because of these factors, investigators have begun to look for more functional and clinically applicable measures to understand the effects of functional ankle instability and the possible cause of the recurrent feeling of giving way during activity.

Kinesthesia and joint position sense are two measures that have been used in an attempt to quantify alterations in sensorimotor function at a joint. Within the last 10 years, investigators have begun to measure both variables in order to understand the sensorimotor effects of functional ankle instability. Throughout the research involving kinesthesia and functional ankle instability, time to detect passive motion and threshold to detect motion are the measures most regularly utilized. The results of these investigations have been inconclusive. Hubbard et al. found no significant differences between healthy and functionally unstable ankles for time to detect passive motion (Hubbard and Kaminski 2002). Refshauge et al. measured plantarflexion and dorsiflexion and found no significant difference in threshold angle to detection of motion between the healthy and functionally unstable ankles (Refshauge, Kilbreath et al. 2000). On the contrary, several studies have found significant differences between healthy and functionally unstable ankle’s threshold to detect motion (Lentell, Baas et al. 1995; Konradsen 1998; Konradsen 2002). Due to the variation in measurement techniques and the reported measures, it is difficult to make a determination about the effect of functional ankle instability on measures of kinesthesia; however, it does appear that threshold to detect motion may be different between healthy and functionally unstable ankles.

Joint position sense is a more commonly used measure of sensorimotor deficits in the functionally unstable ankle however much like kinesthesia the current literature is inconclusive as to its effects. Gross et al. found no significant difference in passive and
active joint position sense measurement in small degrees (10-20 degrees) of inversion and eversion (Gross 1987). However, most studies show a significant alteration in joint position sense with increased replication error within a single trial and over a series of trials in both active and passive movements when comparing functionally unstable to healthy ankles (Gross 1987; Boyle and Negus 1998; Konradsen 1998; Konradsen and Magnusson 2000). It is relatively clear that alterations in joint position sense tend to occur in patients with function ankle instability. While there is no widely accepted hypothesis for why this alteration occurs, in studies with a significant finding, authors tend to believe that de-afferentiation or some alteration in normal sensorimotor activity within the ankle joint is responsible.

Eversion force sense may be related to functional ankle instability. If functionally unstable patients are not as able to sense the amount of force being produced in response to a sudden inversion, they may not be able to produce an appropriate force to counteract an injurious motion. Investigators have shown that at low loads, 10 percent of maximal volitional eversion contraction, a weak positive correlation existed between the number of reported episodes of giving way and eversion force sense (Docherty, Gansneder et al. 2006). However, at loads greater than 10 percent, this correlation was no longer present. The authors suggest that these errors are due to changes in the golgi tendon organ or secondary muscle spindles in response to lateral ankle injury and may be related to the recurrent feeling of instability found in functionally unstable ankles (Docherty, Gansneder et al. 2006). Due to the small amount of research and weak correlation found in this study, it is not possible to state that functional ankle instability is related to low load eversion force sense.
Mechanical joint instability and strength deficits have been investigated in subjects with functional ankle instability. In the investigations of mechanical ligamentous joint instability, there have been mixed results. Early studies showed a limited presence, only 42 percent, of anterior laxity with the anterior drawer test in subjects with functionally unstable ankles (Tropp, Odenrick et al. 1985). Ryan also showed that in a sample of 45 subjects, only 24 percent of subjects with functional ankle instability had a positive talar tilt and roughly 9 percent displayed a positive anterior drawer for inversion and anterior ligamentous laxity respectively (Ryan 1994). A more recent study has shown that a stronger correlation exists in functionally unstable ankles when compared to healthy ankles on both the anterior drawer and talar tilt for anterior and inversion laxity, respectively (Hubbard, Kramer et al. 2007; Hubbard, Kramer et al. 2007). As can be seen in the literature, the link between functional ankle instability and measures of mechanical instability tend to show a weak relationship that cannot be consistently demonstrated. Muscular strength at the ankle is measured in many different ways and has been an area of research regarding functional ankle instability. Most investigations have found no significant differences in strength of the invertors, evertors, plantarflexors or dorsiflexors in functionally unstable ankles when compared to healthy ankles or the uninvolved ankle (Ryan 1994; Lentell, Baas et al. 1995; Kaminski and Hartsell 2002). In these studies strength was assessed via isometric maximal volitional contraction or manual muscle test. Subjects with functional ankle instability were found to have a deficit in eversion peak torque, dorsiflexion peak torque, and eccentric plantarflexor torque through isokinetic strength testing (Tropp 1986; Hubbard, Kramer et al. 2007; Fox, Docherty et al. 2008). While these findings were significant, the lack of consistent findings among those
who found significant differences makes it difficult to generalize results to the functionally unstable ankle population.

The functional and performance effects of functional ankle instability are the most recent area of research in sports medicine and possibly the most clinically applicable, but also the most difficult to make comparisons among. Recent research has utilized several tasks to mimic real life situations in which functional ankle instability may affect performance. Two studies have utilized the Star Excursion Balance Test (SEBT) in order to test postural control in a dynamic monopedal stance, which had previously been shown to be sensitive for detecting functional ankle instability (Olmsted, Garcia et al. 2002; Gribble, Hertel et al. 2004; Gribble, Hertel et al. 2007). The investigators found that functional ankle instability leads to affects on medial, anterior, and posterior SEBT including decreases in maximum reach distance and knee flexion when standing in monopedal stance on the functionally unstable ankle. (Gribble, Hertel et al. 2004; Gribble, Hertel et al. 2007). The relationship between functional ankle instability and performance on various directional hopping tasks when compared to healthy ankles has been studied. A clear positive correlation was found between side hopping, figure-of-8 hop performance, and functional ankle instability; while no significant correlation was found for single hop for distance or the up-down hop. This showed that deficits in a specific functional hopping task may be utilized to help clarify if a subject has functional ankle instability and may also show possible deficits that would cause alteration in functional athletic performance (Docherty, Arnold et al. 2004). Demeritt et al. investigated performance on an agility hopping task in which no significant difference was found in the number of step downs before completion of a hopping course when functionally unstable and healthy ankles were compared. The shuttle run and a co-
contraction task were also measured and no significant difference in time to completion was found between functionally unstable and healthy ankles (Demeritt, Shultz et al. 2002). In the case of functional testing it is apparent that deficits are heavily dependent on the chosen task but tend to be more regularly found in functionally unstable ankles when the tasks are focused on body movement in the frontal plane. While these results are unclear as to the overall effect of functional ankle instability on functional performance they show that more studies related to sport specific tasks should be performed in order to clarify the dynamic relationship between functional movement, athletic performance, and functional ankle instability.

**Muscle Fatigue**

Human muscle fatigue has been defined as a loss of force-generating capacity that may develop due to a variety of internal and external factors at multiple sites along the neuromuscular pathway (Edwards 1981; Edwards 1984; Vollestad 1997). In many cases, this definition has been confused with muscular weakness which has been defined as maintained low maximal force, which persists over long time periods and is exercise independent (Vollestad 1997). While fatigue and weakness are related in some situations, fatigue is an induced condition that only occurs in the presence of exercise of sufficient duration or intensity. Fatigue has been divided into two distinct types within the body, peripheral and central, that have been shown to occur separately depending on the type and duration of fatiguing activity (Edwards 1981; Eberstein and Beattie 1985; Cooke, Franks et al. 1988; Miller, Boska et al. 1988; Macefield, Hagbarth et al. 1991; McKenzie, Bigland-Ritchie et al. 1992; Vollestad 1997; Kent-Braun 1999). Central fatigue has been defined as an impairment
of the central motor drive that can affect alpha motor neurons and their ability to produce action potentials, however the degree to which this affects muscle activation and force output has been largely debated within the literature. A significant amount of central fatigue has been noted following both short and long duration exercise of sub-maximal and maximal intensity in limb and diaphragm musculature of adult subjects (McKenzie, Bigland-Ritchie et al. 1992; Kent-Braun 1999). However, a large criticism of central fatigue literature is the inherent effect of subject motivation on force production and performance during and following fatiguing exercise (Vollestad 1997; Kent-Braun 1999).

Peripheral fatigue is more widely researched throughout both the sports medicine and exercise physiology research with regard to its effect on muscle and functional performance (Edwards 1984; Cooke, Franks et al. 1988; Miller, Boska et al. 1988; McKenzie, Bigland-Ritchie et al. 1992; Baker, Kostov et al. 1993; Johnston, Howard et al. 1998; Kent-Braun 1999). In current literature, peripheral fatigue is defined as metabolic inhibition of the contractile process and excitation contraction failure within a muscle (Kent-Braun 1999). This type of fatigue, while concurrent in some cases, can be produced without inhibition of central pathways and is more likely during normal sustained exercise (Kent-Braun 1999). There have been several proposed pathways to explain the presence of peripheral fatigue including interruption of actin-myosin binding and cycling due to alteration in calcium availability and binding capability, which in turn leads to decreased ability to generate force within contractile tissues. This has been proposed to be related to the increased presence of metabolic byproducts within and surrounding the sarcolemma during fatiguing activity (Sejersted, Hargens et al. 1984; Westerblad, Duty et al. 1993; Vollestad 1997). While peripheral fatigue is widely studied, it is hard to study differences in muscle performance and
force production due to the different contractile and energetic properties of fast twitch versus slow twitch fiber types. Long duration submaximal exercise has been shown to affect excitation-contraction coupling while high intensity exercise has been shown to cause an accumulation of metabolic byproducts within the sarcolema (Edwards 1981; Edwards 1984; Cooke, Franks et al. 1988; Miller, Boska et al. 1988; Baker, Kostov et al. 1993). While peripheral and central fatigue occur through discreet pathways recent studies have looked at their interaction and individual contributions to overall performance deficits during maximal and sub-maximal activity with relatively consistent results. In most studies, during maximal contraction exercise and submaximal contraction exercise, peripheral fatigue was found to be responsible for a large portion of overall loss of force production capability (Macefield, Hagbarth et al. 1991; McKenzie, Bigland-Ritchie et al. 1992; Kent-Braun 1999). In one study, this ratio was found to be as high as 80 percent peripheral fatigue, measured through metabolic byproducts and direct muscle stimulation, as compared to 20 percent central fatigue, measured through direct alpha motor neuron stimulation and iEMG measurement during volitional activity (Kent-Braun 1999). This suggests that peripheral fatigue is responsible for a larger portion of functional deficits seen in healthy adults (McKenzie, Bigland-Ritchie et al. 1992; Kent-Braun 1999).

There are many ways to measure human muscle fatigue during isometric, isokinetic, and functional tasks. The most common manner of measurement is a comparison of maximal volitional force output in a desired motion to a force output during a sustained or repeated contraction. This method is widely used and has been applied to concentric, eccentric, and isometric contraction of lower extremity muscles including ankle invertors, evertors, plantarflexors, and dorsiflexors (Edwards 1981; Edwards 1984; Eberstein and
Beattie 1985; Baker, Kostov et al. 1993; Ng, Richardson et al. 1996; Vollestad 1997; Johnston, Howard et al. 1998; Svantesson, Osterberg et al. 1998; Kent-Braun 1999; Forestier, Teasdale et al. 2002; Yaggio and McGregor 2002; Harkins, Mattacola et al. 2005; Jackson, Gutierrez et al. 2007; Wilson and Madigan 2007). While this method is widely utilized and accepted, little research has been performed to clarify what percentage or duration of force production decrease denotes a fatigued muscle and for how long the muscle will remain fatigued. Several studies have compared sustained isometrics, totally concentric, concentric-eccentric, and functional protocols and their ability to produce measurable force production deficits over a period of time (Leisman, Zenhausern et al. 1995; Svantesson, Osterberg et al. 1998; Kent-Braun 1999; Kollmitzer, Ebenbichler et al. 1999; Ebenbichler, Bonato et al. 2002; Gribble, Hertel et al. 2004; Gribble, Hertel et al. 2007).

While results have varied depending on the testing procedure utilized, it is clear that functional tasks are able to produce the greatest sustained deficits in force production. Totally concentric and concentric-eccentric protocols have also been studied in depth and it is clear that both are able to provide fatigue to a level of 50 percent maximal volitional contraction however, in the case of totally concentric these alterations have been shown to last as little as 40 seconds and in concentric eccentric situation, fatigue may last as little 1-2 minutes (Vollestad 1997; Svantesson, Osterberg et al. 1998; Kent-Braun 1999; Kollmitzer, Ebenbichler et al. 1999; Harkins, Mattacola et al. 2005). Harkins et al. found that 30 percent maximal volitional contraction via totally concentric protocol in the plantarflexors and dorsiflexors of healthy adults elicits fatigue lasting roughly 75 seconds when compared to 35 seconds under the 50 percent maximal volitional contraction condition (Harkins, Mattacola et
These limitations have made it difficult to measure the effects of fatigue during tasks due to the inability to insure that presence of fatigue during testing.

Recent research has explored sustained hold isometric fatigue protocols as an alternative to more traditional totally concentric or concentric-eccentric protocols. Several studies have examined protocols that utilize a sustained isometric contraction that is maintained at 20% of maximum voluntary isometric contraction until failure. Failure has not been defined consistently throughout the literature, however it is mostly commonly shown to be a decrease in force production for a time greater than 5 seconds (Hunter and Enoka 2003; Hunter, Critchlow et al. 2004; Hunter, Critchlow et al. 2005; Hunter, Rochette et al. 2005; Enoka and Duchateau 2008). These studies have shown significant decreases in force production in varied subject age groups as well as consistent variations in EMG activity within those groups. While these protocols have been largely studied in the upper extremity, they offer a promising alternative to more traditional fatigue protocols which may allow for a longer lasting fatigue that more closely mirrors that of functional activity.

The limitations in maximal volitional contraction comparison as a measure of fatigue has caused sports medicine researchers to utilize alternative methods in order to ensure fatigue during both open and closed kinetic chain activity. Among these measurements, the two most common are increased EMG amplitude and spectral shift in EMG mean and median power frequency. An increase in EMG amplitude has been shown to be associated with fatigue when compared to both functional performance and decrease in maximal force output. It is theorized that as muscle becomes fatigued, in order to maintain a desired level of force output, motor unit recruitment must increase. Due to this increase in motor unit recruitment, an increase in EMG amplitude is seen (Edwards 1981; Edwards 1984;
Macefield, Hagbarth et al. 1991; Vollestad 1997). While EMG amplitude increase has been shown to be somewhat reliable for measurement of fatigue, some researchers have found that accurate measurement is highly dependent on experimental setup and the presence of both background noise and EMG crosstalk. Because of these limitations, it may be difficult to apply an increase in EMG amplitude as a measure of fatigue in more functional activities such as lifting and lunging (Vollestad 1997; Kollmitzer, Ebenbichler et al. 1999; Ebenbichler, Bonato et al. 2002).

Spectral shift in mean and median power frequency are widely used measures of fatigue that have been shown to be reliable and valid in the lower extremity musculature of healthy adults (Vollestad 1997; Svantesson, Osterberg et al. 1998; Kollmitzer, Ebenbichler et al. 1999). It is hypothesized that a decrease in mean or median power frequency during sustained activity is a result of decreased conduction velocity and frequency of excitation of motor neurons due to an accumulation of local metabolic products (Edwards 1981; Eberstein and Beattie 1985; Vollestad 1997). In several studies involving isokinetic fatigue protocols in the lower extremity of healthy adults, a negative spectral shift has been shown during fatigue with a return to normal mean and median power frequency upon return to normal force output (Eberstein and Beattie 1985; Vollestad 1997; Kent-Braun 1999). While there are some investigators who believe these measurements are too heavily dependent on experimental setup and may be largely affected by EMG background noise and possible motion artifact, evidence tends to show that median or mean power frequency is a valid measure to confirm muscle fatigue when force production deficits are present (Edwards 1981; Edwards 1984; Eberstein and Beattie 1985; Macefield, Hagbarth et al. 1991; Vollestad 1997; Kent-Braun 1999; Ebenbichler, Bonato et al. 2002).
Fatigue has been shown to have varied effects on several physiological and functional measures in both healthy and functionally unstable ankles. The effects of fatigue on peroneal EMG latency has been studied in healthy adults and in both studies, isokinetic concentric-eccentric fatigue has been found to cause no significant difference in pre-fatigue and post-fatigue values of EMG latency (Jackson, Gutierrez et al. 2007; Wilson and Madigan 2007). These findings, like much of the research on peroneal reflex reactions, are incomplete, however because there is no discussion of electromechanical delay. The results are therefore difficult to apply to functional activity. Without knowing if fatigue affects the ability of the peroneals to produce force in response to a sudden inversion perturbation, it is not possible to clarify the functional differences caused by fatigue during sudden ankle inversion (Jackson, Gutierrez et al. 2007; Wilson and Madigan 2007). Postural control during balance tasks such as the Star Excursion Balance Task has also been studied in subjects who have been fatigued via a concentric-concentric Biodex protocol and a more functional lunge protocol focusing on the ankle plantarflexors and dorsiflexors. In both studies, a significant decrease in maximal reach distance and peak knee flexion angle was seen in subjects when pre-post fatigue measurements were compared (Gribble, Hertel et al. 2004; Gribble, Hertel et al. 2007). Postural sway velocity has been shown to be significantly affected by concentric-concentric fatigue of the plantarflexors and dorsiflexors of the ankle to both 30 and 50 percent of maximal volitional contraction. While deficits lasted significantly longer in subjects fatigued to 30 percent of maximal volitional contraction, significant functional deficits were seen in both groups (Harkins, Mattacola et al. 2005). The physiological and functional deficits shown in these studies have shown that fatigue can have significant effects on both healthy and functionally unstable ankles during both functional and non-functional
tasks. Very little investigation has been done to gain a better understanding into what is responsible for baseline stability at the ankle and if it is affected by functional fatigue. Hunter et al. has shown that there is an invariance in ankle stiffness in healthy subjects following stimulated isometric fatigue to 50 percent of maximal volitional contraction, however no research has examined the effects of fatigue in functionally unstable ankles (Hunter and Kearney 1983). While functional measures and physiological responses are important for gaining a better understanding of functional ankle instability, these measures do not address that basis of ankle stability and the alterations that this functional ankle instability causes in the intrinsic stability and stiffness of the ankle joint.

**Ankle Stiffness**

Loss of stability or the ability of a system to return to its original state after a disturbance is proposed to be the primary cause of joint injury (Wagner and Blickhan 1999; Docherty, Arnold et al. 2004). In humans, self stabilization occurs on a constant basis via intrinsic tissue properties as well as feedback and feed forward response to perturbations. This combination of active and passive tissue response to perturbation is known as stiffness (Duan, Allen et al. 1997; Wagner and Blickhan 1999; Blackburn, Padua et al. 2006; Zinder, Granata et al. 2007). Stiffness is the ability of a tissue or joint to resist a change in length or angle respectively (Wagner and Blickhan 1999). Currently, most ankle stiffness research is done in regard to perturbations in the sagittal plane and is therefore dependent on the properties of the dorsiflexors and plantarflexors of the ankle (Hunter and Kearney 1982; Kearney and Hunter 1982; Hunter and Kearney 1983; Allum and Mauritz 1984; Sinkjaer, Toft et al. 1988; Weiss, Hunter et al. 1988; Duan, Allen et al. 1997; Kearney, Stein et al. 2007).
1997; Kirsch and Kearney 1997; Wagner and Blickhan 1999; Mirbagheri, Barbeau et al. 2000; Maas, Baan et al. 2001; Loram and Lakie 2002; Docherty, Arnold et al. 2004; Loram, Maganaris et al. 2004; Casadio, Morasso et al. 2005; Padua, Carcia et al. 2005; Blackburn, Padua et al. 2006; Loram, Maganaris et al. 2007; Loram, Maganaris et al. 2007; Ludvig and Kearney 2007). In these experiments, the human body is modeled as an inverted pendulum, similar to a metronome, with an axis of rotation about the ankle in the sagittal plane and torque about the axis that is equal to the force of body weight at a distance equal to body height. It is hypothesized that ankle stiffness acts as the primary restraint, in varied degrees, to sagittal plane motion about this axis of rotation both during standing and locomotion (Hunter and Kearney 1982; Kearney and Hunter 1982; Hunter and Kearney 1983; Sinkjaer, Toft et al. 1988; Sinkjaer, Nielsen et al. 1995; Kearney, Stein et al. 1997; Kirsch and Kearney 1997; Wagner and Blickhan 1999; Loram, Kelly et al. 2001; Loram and Lakie 2002; Loram and Lakie 2002; Casadio, Morasso et al. 2005; Loram, Maganaris et al. 2007; Loram, Maganaris et al. 2007). This hypothesis has been widely studied in both conditions and shown to be only partially true. Stiffness is composed of several factors which can be described as active and passive components. Passive components are thought to be made up of tissue and joint properties that act as restraints to motion without reflex activation. This includes passive joint congruency and the elastic components that are inherent to skeletal muscle. While these components have been shown to be consistent throughout the healthy population, they are able to produce enough force to effectively stabilize the ankle joint during standing, perturbation, or locomotion. Several studies have been performed in standing with gradual small amplitude motions in the sagittal plane to show the relative ability of passive stabilizers to produce enough force to overcome perturbation. These
studies have shown that any deviation outside of neutral joint alignment produces enough force to overcome the capacity of passive restraints (Hunter and Kearney 1982; Kirsch and Kearney 1997; Casadio, Morasso et al. 2005; Loram, Maganaris et al. 2007; Loram, Maganaris et al. 2007). In small amplitude motion under 3 degrees in the sagittal plane, up to 70 percent of stabilizing force has been shown to be produced via passive restraints, however in large perturbations of up to 7 degrees as much as 85 percent of stabilization was not able to be explained via passive restraints (Loram, Maganaris et al. 2007; Loram, Maganaris et al. 2007). It is theorized that while passive components of muscle stiffness are insufficient to prevent instability at the ankle joint, active components such as stretch reflex and muscle activation are sufficient to overcome the remaining torque about the ankle during low velocity perturbation (Hunter and Kearney 1982; Allum and Mauritz 1984; Sinkjaer, Toft et al. 1988; Weiss, Hunter et al. 1988; Kearney, Stein et al. 1997; Kirsch and Kearney 1997; Mirbagheri, Barbeau et al. 2000; Loram, Kelly et al. 2001; Loram and Lakie 2002; Loram and Lakie 2002; Casadio, Morasso et al. 2005; Loram, Maganaris et al. 2007; Loram, Maganaris et al. 2007; Ludvig and Kearney 2007).

The relative contributions to ankle stiffness in the sagittal plane of passive and active components have been investigated in standing and hopping (Padua, Garcia et al. 2005). While it is clear that contributions to stiffness are altered by varied angle and velocities of perturbation, with larger active contributions to sagittal plane stability in large angles and high velocity perturbations at the ankle, it is unclear how these contributions vary during functional activity (Hunter and Kearney 1982; Kearney and Hunter 1982; Weiss, Hunter et al. 1988; Loram, Maganaris et al. 2007; Loram, Maganaris et al. 2007). Hunter et al investigated the effects of induced fatigue to 50 percent of maximal volitional contraction of
the plantarflexors and dorsiflexors in order to measure the change in stiffness at the ankle joint that followed. According to this study no significant change in stiffness was found during varied sagittal plane perturbations (Hunter and Kearney 1982; Hunter and Kearney 1983). Sagittal plane research has been very helpful in understanding the overall activity of the ankle during normal sway movement, posture, and gait, however most ankle injuries occur in the frontal plane (Freeman, Dean et al. 1965; Birrer, Fani-Salek et al. 1999). Currently, there is a lack of research related to inversion-eversion perturbation and the ability of the ankle to produce passive and active stiffness in response (Zinder, Granata et al. 2007). While this is due to difficulties in modeling the ankle in the frontal plane and a lack of viable measurement devices, reference values for stiffness and the relationship between stiffness and proprioception have been established. Baseline stiffness measures on an inversion-eversion oscillation cradle in the frontal plane have been compared to error in joint position sense and force sense with no significant correlation (Docherty, Arnold et al. 2004; Zinder, Granata et al. 2007). This lack of research on stiffness in the frontal plane has made it difficult to understand the possible relationship between ankle stiffness and functional ankle instability (Zinder, Granata et al. 2007).

**Conclusion**

Functional ankle instability clearly causes alterations in both physiologic response to perturbation and functional performance on some tasks at the ankle. While current research has a widely varied definition of functional ankle instability and there are no consistent inclusion criteria throughout the literature, it has become obvious that more research must be done in order to better understand the scope of effects on the ankle. Muscle fatigue and ankle
stiffness have both been studied in great depth however in both cases, the volume of research related to motion in the frontal plane and possible application to functional instability is quite limited. Because of these gaps in the literature it is our goal to show the potential affects of functional fatigue on frontal plane ankle stiffness in both healthy and functionally unstable ankles. By measuring stiffness under these conditions we hope to better understand one of the factors that may lead subjects with functional ankle instability to report the subjective feeling of “giving way” during functional activity as well as to understand why active adults are at greater risk for ankle injury during activity. It has been proposed that a lack of stability is the greatest predisposition to joint injury. If this is true, then an alteration in frontal plane ankle stiffness due to fatigue or functional ankle instability could undoubtedly be a key to understanding why some patients have a recurrent feeling of ankle instability following acute ankle injury while others do not.


Chapter 3

Methods

Subjects

Forty physically active males and females between the ages of 18-30 were recruited from the population of the University of North Carolina at Chapel Hill. Subjects were recruited through mass email and verbally in activity classes. Physically active was defined as participating in recreational, club, or varsity athletics at least 3 times a week for at least 30 minutes per session. Subjects had no history of severe lower extremity injury that required physician’s assessment within six months of testing, no current symptoms of an acute ankle sprain, no history of ankle fracture, no current participation in physical therapy, and no history of concussion or neuromuscular dysfunction. Subjects were stratified into two groups of equal numbers; those with functionally stable and those with functionally unstable ankles. Subjects were included in the functional instability group if they had a history of ankle sprain to their dominant or non-dominant side that resulted in a score above five on the dichotomous questions of the AII (Docherty, Arnold et al. 2004).

Instrumentation

Ankle Instability Instrument

Functional ankle instability was defined as a subjective feeling of recurrent giving way during daily functional activity following an initial ankle sprain. Screening for
functional ankle instability occurred via the AII which has been demonstrated as a valid and reliable indicator of functional ankle instability (Docherty, Gansneder et al. 2006). A cutoff parameter for the AII has been establish as a “yes” answer to question #1 as well as a “yes” answer to no less than four other yes-no questions. The free response portions of the AII were used in stratifying the groups in this study (Appendix B).

Electromyography

Electromyography (EMG) was used to measure muscle activity of the peroneus longus. The Delsys Bagnoli-8 hard-wired EMG system (Boston, MA) was used, with differential amplification using an 8 channel amplifier. The EMG signal was amplified and passed through an A/D converter (National Instruments, Austin, Texas) sampling at 1000 Hertz. Raw EMG data were collected using DataPac v. 2k2 software (Run Technologies, Mission Viejo, California).

The skin was prepared by shaving the area of maximal bulk for each muscle, cleaning the area with alcohol, and lightly abrading the area to ensure good electrode contact and transmission. A bar Ag/AgCl surface electrode (Delsys Inc., Boston, MA) was fixed onto the point of maximal muscle girth of the peroneus longus muscle belly with two bars lying parallel to the muscle fibers. EMG electrodes were fixed using adhesive collars and athletic tape. A reference electrode was placed over the anterior tibia. Manual muscle tests were used to ensure minimal noise and proper electrode placement.

Inversion-Eversion Swaying Cradle

Ankle stiffness was measured via an inversion-eversion swaying cradle device (Figure 1) that has been demonstrated as valid and reliable (Zinder, Granata et al. 2007). Ankle stiffness was measured with the subject in a seated position with the hip and knee at
90 degrees of flexion and the ankle in a neutral position. An axial load equal to fifty percent body mass was positioned on the shank directly above the ankle being tested. Trial-to-trial reliability (ICC_{2,1}) has been shown to be 0.96 with an SEM of 2.05 Nm/rad, and day-to-day reliability (ICC_{2,k}) has been shown to be 0.93 and an SEM of 3.00 Nm/rad (Zinder, Granata et al. 2007). Cradle angular displacement was recorded with a 270° single turn potentiometer (Clarostat, Mexico City, Mexico) that was aligned with the cradle’s axis of rotation.

**Load Cell**

Peroneus longus fatigue was produced via a sustained hold eversion fatigue protocol using a 500 lbs. load cell (Honeywell, Golden Valley, Minnesota) bench apparatus. This apparatus was secured to a padded treatment table in the Sports Medicine Research Laboratory (Figure 2). A small rubber button was secured to the load cell in order to allow subject to apply compressive force to the load cell via ankle eversion. Force output was monitored via a custom Labview (National Instruments, Austin, Texas) program with a visual display on a nearby computer monitor.

**Procedure**

Subjects reported to the Sports Medicine Research Laboratory (FG 06F) for testing sessions. Inclusion and exclusion criteria were confirmed and subjects were required to read
and sign an Institutional Review Board approved informed consent document. The AII was completed by all subjects and used to assign experimental groups. Subjects without history of ankle sprain and below the cutoff score for the AII were assigned to the control group. Subjects in the functionally stable ankle group were matched to subjects in the functionally unstable group based on dominance of the functionally unstable ankle. Limb dominance was defined as the leg with which the subject would kick a ball for maximum distance. The primary investigator (CK) was responsible for group assignment. Subjects were asked to contact the investigators if their injury status changed before their testing session.

The skin over the maximum bulk of the peroneus longus was shaved, lightly abraded, and swabbed with alcohol after which EMG electrodes were affixed with adhesive collars. Subjects were asked to remove their shoes. Peroneus longus EMG activity was recorded for three maximum volitional isometric contractions (MVICs) lasting five seconds each via isometric eversion on the load cell apparatus. The subjects were then seated with their foot in the inversion-eversion cradle for stiffness assessment. The subject’s foot was positioned so that the midline of the cradle aligned with the subjects’ second ray. The subject’s hip and knee were positioned in 90 degrees of flexion, and a weight equaling fifty percent of the subject’s body mass was positioned directly over the ankle being tested as described in Zinder, et al (Figure 1). Subjects were instructed to close their eyes and remain completely relaxed for the duration of each trial. Each trial began with a ball being dropped from a constant height of 100cm in order to initiate inversion-eversion sway of the cradle. The ball was dropped on the side of the cradle corresponding with the lateral side of the subject’s ankle. EMG data were recorded for 250ms prior to and 3000ms following ball contact with the cradle device. Five trials were collected for analysis. Peroneus longus mean EMG pre-
activation and mean amplitude were measured for each trial. EMG pre-activation was
defined as the mean EMG amplitude 250ms prior to initiation of perturbation on the
inversion-eversion swaying cradle device. Peroneus longus mean EMG amplitude was
defined as the mean EMG amplitude for 500ms following perturbation of the
inversion/eversion swaying ankle cradle.

The sustained hold isometric fatigue protocol was completed following the baseline
ankle stiffness measurements. Subjects were seated on the load cell apparatus supported by a
standard treatment table. The subjects’ shanks were secured to the apparatus with a strap to
prevent hip internal rotation and a foam block to prevent hip abduction. The subjects
positioned their foot in the position of greatest comfort with the base of their fifth metatarsal
in contact with the button extending from the load cell (Figure 2). The subjects were
instructed to push as hard as they could throughout all trials and were given verbal cues such
as “keep going” and “push harder.” The subjects were asked to complete one five-second
eversion MVICs in order to establish peak isometric eversion force output (N). Subjects
were then asked to maintain an eversion isometric contraction at a force equal to 20% of
MVIC which was monitored visually on a custom Labview program on a nearby computer
monitor. Fatigue was achieved when eversion force fell below 10% of MVIC for five
consecutive seconds (Hunter, Rochette et al. 2005). Typically, fatigue was achieved after 15-
20 minutes of sustained hold isometric eversion. Immediately following completion of the
fatigue protocol, the subjects were repositioned on the inversion-eversion cradle and five
trials were used to re-measure ankle stiffness with procedures as described above.

Data Reduction
Electromyography

Raw EMG data were rectified, smoothed and filtered prior to processing. Data were smoothed via a root mean squared 20 millisecond sliding window function, and filtered via 4th order Butterworth band pass filter (20-350Hz) in a custom Matlab program.

Calculation of Ankle Inertia and Stiffness

In order to calculate ankle stiffness we first measured the rotational inertia of the system by adding known amounts of mass at a fixed distance from the cradle’s axis of rotation. A regression analysis was then used to calculate ankle stiffness. In order to calculate the inertia of the system zero, one, and two weights of equal magnitude (0.57 kg) were added at the same distance from the axis of rotation to each side of the cradle device. Damped frequency ($\omega_d$) of the system was measured with a potentiometer. Using the collected data, the natural frequency of oscillation and the external inertia of the system were calculated. Stiffness of the system was defined as the slope of the regression line between the natural frequency of oscillation and the applied external inertia. Assuming second-order dynamical behavior, the formula:

$$I_{Ext} = \frac{(k + mgl)}{\omega_n^2} - I_0 \quad \text{or} \quad I_{Ext} = (k + mgl)\left(\frac{1}{\omega_n^2}\right) - I_0$$

allowed us to plot the added external inertia ($I_{Ext}$) versus the inverse of the square of the natural frequency ($1/\omega_n^2$). The natural frequency of the oscillations ($\omega_n$) was determined by measuring the damped frequency of the oscillations and combining it with the damping coefficient ($\beta$) which must be included due to its affect on the magnitude of the natural frequency of oscillation. It was determined that the pendulum behavior (ml - mass of the system x gravitational acceleration x length of the radius) had an effect of less than 1% on the ankle stiffness, and will be therefore ignored making the equation resemble the equation
of a line \( y = mx + b \). The stiffness \( k \) is the slope of the regression line and the inertia of the ankle and cradle is the intercept \( I_0 \) (Wagner and Blickhan 1999; Zinder, Granata et al. 2007).

**Statistical Analysis**

An a priori level of \( P<0.05 \) was set as the level of significance for all statistical analyses. Three separate 2 (pre-fatigue, post-fatigue) x 2 (stable ankle, functionally unstable ankle) mixed model repeated measures ANOVAs were used to analyze ankle stiffness, peroneus longus mean EMG pre-activation, and peroneus longus mean EMG amplitude between groups at baseline and post-fatigue. Post-hoc analysis was completed using a Bonferroni adjustment for multiple t-tests. Statistical analyses were completed in SPSS version 15.0 for the PC.
Chapter 4

Results

Forty subjects (14 men and 26 women, age = 21.7 ± 2.5 years, weight = 173.3 ± 11.0 kilograms, height = 74.7 ± 20.9 centimeters) were tested in this study (Table 1).

Table 1. Subject Demographics

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Age (years)</th>
<th>Height (cm)</th>
<th>Weight (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable</td>
<td>20</td>
<td>21.5 ± 2.3</td>
<td>173.2 ± 10.3</td>
<td>69.2 ± 11.4</td>
</tr>
<tr>
<td>Functionally Unstable</td>
<td>20</td>
<td>22.0 ± 2.7</td>
<td>173.5 ± 12.0</td>
<td>80.1 ± 26.6</td>
</tr>
<tr>
<td>Total</td>
<td>40</td>
<td>21.7 ± 2.5</td>
<td>173.3 ± 11.0</td>
<td>74.7 ± 20.9</td>
</tr>
</tbody>
</table>

a Values are means ± standard deviations

Ankle Stiffness

A 2 (pre-fatigue, post-fatigue) x 2 (stable ankle, functionally unstable ankle) mixed model repeated measures ANOVA was calculated for ankle stiffness (Table 2). There were no significant main effects for fatigue status [F(1,38) = 0.44, P = 0.51] or group [F(1,38) = 1.16, P = 0.29], and there was no significant interaction between fatigue status and group [F(1,38) = 1.95, P = 0.17].

Table 2. Ankle Stiffness (Nm/rad)

<table>
<thead>
<tr>
<th></th>
<th>Pre-fatigue</th>
<th>Post-fatigue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable</td>
<td>38.71 ± 12.73</td>
<td>35.63 ± 10.22</td>
</tr>
<tr>
<td>Functionally Unstable</td>
<td>41.43 ± 17.63</td>
<td>42.53 ± 17.63</td>
</tr>
</tbody>
</table>

a Values are means ± standard deviations
**Electromyographical Data**

A 2 (pre-fatigue, post-fatigue) x 2 (stable ankle, functionally unstable ankle) mixed model repeated measures ANOVA was calculated for peroneus longus mean EMG pre-activation (Table 2). There was a significant main effect for fatigue status [$F(1,38) = 4.67, P = .04$] with pre-fatigue EMG activity ($15.97 \pm 16.13 \% \text{MVIC}$) greater than post-fatigue EMG activity ($11.72 \pm 15.45 \% \text{MVIC}$). There was no significant main effect for group [$F(1,38) = 2.24, P = 0.14$] and there no significant interaction effect between fatigue status and groups [$F(1,38) = 0.45, P = 0.51$].

**Table 3. Peroneus Longus EMG Pre-activation (\%MVIC)$^a$**

<table>
<thead>
<tr>
<th></th>
<th>Pre-fatigue</th>
<th>Post-fatigue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stable</td>
<td>18.70 ± 19.47</td>
<td>15.76 ± 20.88</td>
</tr>
<tr>
<td>Functionally Unstable</td>
<td>13.25 ± 11.80</td>
<td>7.67 ± 4.42</td>
</tr>
</tbody>
</table>

$^a$Values are means ± standard deviations

A 2 (pre-fatigue, post-fatigue) x 2 (stable ankle, functionally unstable ankle) mixed model repeated measures ANOVA was calculated for peroneus longus mean EMG amplitude (Table 4). There was a significant main effect for fatigue status [$F(1,38) = 7.22, P = .01$] with pre-fatigue EMG activity ($28.43 \pm 25.58 \% \text{MVIC}$) greater than post-fatigue EMG activity ($20.92 \pm 20.63 \% \text{MVIC}$). There was no significant main effect for group [$F(1,38) = .796, P = .39$] and no significant interaction effect between fatigue status and groups [$F(1,38) = 1.47, P = .23$].
<table>
<thead>
<tr>
<th>Stable</th>
<th>Pre-fatigue</th>
<th>Post-fatigue</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>29.77 ± 23.17</td>
<td>25.65 ± 23.78</td>
</tr>
<tr>
<td>Functionally Unstable</td>
<td>27.09 ± 28.32</td>
<td>16.19 ± 16.16</td>
</tr>
</tbody>
</table>

<sup>a</sup> Values are means ± standard deviations
Chapter 5

Discussion

The goal of this study was to better understand the effects of fatigue on ankle stability in both stable and functionally unstable ankles. Through the use of a sustained hold isometric fatigue protocol, we attempted to induce a long lasting peripheral fatigue of the peroneus longus in order to measure the effects fatigue would have on ankle stiffness, a direct measure of ankle joint stability. Therefore, the most important finding in this study was that we found no difference in ankle stiffness regardless of group or fatigue status. This finding is consistent with our research hypotheses regarding ankle stiffness however, the lack of difference in stiffness relative to both fatigue status and ankle stability may seem counterintuitive due to their respective definitions (Wagner and Blickhan 1999).

Stiffness is utilized as a measure of joint stability. It is a multi-factorial measure that takes into account muscle activity, load applied to the joint, and passive joint restraints such as ligamentous, capsular, and boney structures that may act to resist joint translations (Duan, Allen et al. 1997; Wagner and Blickhan 1999; Blackburn, Padua et al. 2006; Zinder, Granata et al. 2007). However, in previous studies that have looked into the effect of functional ankle stability on ankle stiffness, no difference has been seen between stable and functionally unstable ankles (Santos and Liu 2008). While there are several potential reasons for this lack of difference, including muscle pre-activation differences and possible neurological compensation, current unpublished investigations in our laboratory have shown that the position in which the ankle is tested may hold the answer. In this investigation, it has been
shown that when ankle stiffness is compared in stable and functionally unstable ankles with the ankle positioned in a neutral position, no difference is seen between the groups. However, in a separate investigation in our laboratory regarding the effects of sagittal plane ankle positioning on ankle stiffness, preliminary data shows a potential difference between stable ankles and functionally unstable ankles when positioned in plantarflexion during stiffness testing. The cause of these findings requires further study, but it is hypothesized that much like the common ankle sprain, the ankle is more vulnerable in plantarflexion and therefore due to less boney congruency and insufficient soft tissue stabilization may show a decrease in joint stiffness (Hertel 2002). In the case of this study, the fact the testing was completed in a neutral joint position may have limited our ability to see potential changes in ankle stiffness both between groups and across fatigue status.

Our results also showed a difference in peroneus longus pre-activation and mean EMG activity when pre-fatigue and post-fatigue measurements were compared. While there were no other statistically significant results, when looking at 95% confidence intervals, it appears that there may be a tendency toward post-fatigue functionally unstable ankle subject to show lower values for both EMG variables. These findings are somewhat consistent with previous literature and may begin to explain why no difference in ankle stiffness was found (Wilson and Madigan 2007). No variations in EMG amplitude or reflex amplitude have been seen consistently during sudden inversion testing when stable and functionally unstable ankles have been compared (Bosien, Staples et al. 1955; Freeman, Dean et al. 1965; Konradsen and Ravn 1990; Konradsen, Voigt et al. 1997; Hertel 2000; Beynnon, Murphy et al. 2002; Vaes, Duquet et al. 2002; McVey, Palmieri et al. 2005). However, when a fatigue protocol has been added to the testing protocol involving stable ankles, a decrease in mean
EMG amplitude was found when pre-fatigue values were compared to post-fatigue values, consistent with our findings (Wilson and Madigan 2007). To our knowledge, no investigation into both pre-activation and mean EMG amplitude during sudden inversion has been completed, but the consistency of past findings with regard to the lack of difference in mean EMG amplitude between groups in combination with a decrease in post-fatigue values compared to pre-fatigue values shows our findings to be in line with previous literature (Lynch, Eklund et al. 1996; Konradsen, Voigt et al. 1997; Benesch, Putz et al. 2000; Fernandes, Allison et al. 2000; Konradsen, Peura et al. 2005). While this does not directly explain the lack of difference in ankle stiffness, in several of the studies where no difference in mean EMG amplitude was found between stable and functionally unstable ankles, time to peak torque and electromechanical delay were also measured (Lynch, Eklund et al. 1996; Konradsen, Voigt et al. 1997; Benesch, Putz et al. 2000; Fernandes, Allison et al. 2000; Konradsen, Peura et al. 2005). Results in these studies were highly variable and showed little clinically significant difference between groups (Lynch, Eklund et al. 1996; Konradsen, Voigt et al. 1997; Benesch, Putz et al. 2000; Fernandes, Allison et al. 2000; Konradsen, Peura et al. 2005). When no change in mean EMG and no change in time to force generation are considered together, it becomes clearer that the lack of change in activation and force generation of the peroneals, may contribute to the lack of difference in stiffness between stable and functionally unstable ankles.

There are several plausible hypotheses that have been utilized to explain the alteration in pre-activation and mean EMG amplitude following fatigue. These include an alteration in muscle membrane excitability, which may lead to an increase or decrease in neural activity to elicit a contraction, and selective fiber type fatigue within a muscle dependent on the
duration and force demands of the fatiguing activity (Hunter, Yoon et al. 2008). In the present study, there is potential that both a decrease in neural drive and selective fiber type played a role in the significant decrease in both EMG variables over time as well as the lack of significant difference ankle joint stiffness seen in this study (Yang Jin-Chul 1986; Hunter, Yoon et al. 2008).

Despite the significant decrease in both EMG variables following fatigue, it remains unclear whether the sustained hold fatigue protocol utilized in this study was sufficiently taxing to fatigue the predominantly fatigue resistant peroneus longus. In preparation for this investigation, it was our goal to identify a fatigue protocol that would allow for a long lasting localized fatigue of the peroneus longus. While most current investigations employ a concentric-eccentric based fatigue protocol with high force output in both contraction types, it has been repeatedly shown that this fatigue tends to be short lived (75 seconds) and peripheral in nature (Harkins, Mattacola et al. 2005). These factors may not have allowed enough time for post-fatigue stiffness testing to occur while the subject remained sufficiently fatigued and therefore may have affected the result of this investigation. Therefore, choosing a 20% MVIC isometric sustained hold fatigue protocol, allowed us greater confidence in both the effectiveness and duration of fatigue of the peroneus longus muscle.

While the validity and cause of spectral shift is debated in the literature, these findings support our theory regarding the adequacy and specificity of the fatigue induced in adults (Vollestad 1997; Svantesson, Osterberg et al. 1998; Kollmitzer, Ebenbichler et al. 1999). Due to the small percentage of maximal force required (20%) and long duration of contraction (15-20 minutes), it is plausible that the peroneus longus, which is predominantly composed of type I and type IIa fatigue resistant muscle fibers, was not appropriately
fatigued (Yang Jin-Chul 1986). Throughout the literature, it is clear that in most fatigue protocols, type IIb muscle fibers are least resistant to fatigue especially in cases of long duration contraction with both constant and varied loads (Edwards 1981; Kent-Braun 1999; Dimitrova and Dimitrov 2003; Enoka and Duchateau 2008; Hunter, Yoon et al. 2008). The rapid fatigue of type IIb fibers may lead to an alteration in force generating capacity over time, but it is unclear what effect this may have on the characteristics of EMG activity recorded both during and following the fatiguing activity. The single fiber muscle EMG activity of type IIb fibers has been previously investigated in muscles with similar fiber distributions to that of peroneus longus and has shown that while at rest type IIb fibers tend to have smaller EMG amplitude than type I and IIa fibers. During activity they exhibit higher amplitude EMG activity and high amplitude neural bursting behavior as they reach fatigue (Yang Jin-Chul 1986; Hunter, Critchlow et al. 2004; Hunter, Critchlow et al. 2005; Hunter, Yoon et al. 2008). Both of these factors lead to an increase in mean EMG when compared to more fatigue resistant fibers. Therefore, if the fatigue protocol utilized in this study did not implement sufficient load or duration to fatigue the type I and type IIa fibers in the peroneus longus, it is possible that the significant decrease in mean EMG amplitude was driven by the decrease in type I fiber activity due to selective fatigue and not a more global muscle fatigue.

Current literature on the kinetics of gait and posture have shown that the force production demands on the peroneus longus throughout functional activity are highly variable, but in many cases exceed 20 percent of MVIC during repetitive motions (Delahunt, Monaghan et al. 2006; Ty Hopkins, McLoda et al. 2007). This fact combined with the histological characteristics of the peroneus longus leads us to believe that 20 percent of
MVIC may not have been a sufficient load to allow for some level of central fatigue coupled with fatigue of fast twitch type IIb muscle fibers. This low percentage of MVIC may not have allowed us to see the true effects that a fatigue protocol, modeled after functional demands on the muscle, may have had on ankle stiffness. This shortcoming may be confirmed by median power frequency data collected during testing. No spectral shift from baseline to post-fatigue conditions was found, which may show insufficient peripheral fatigue to have an effect on the force generating potential of the peroneus longus.

The ankle joint is a complex system that requires coordinated activation of several muscles in order to effectively complete the multi-planar motions needed for gait and other functional activities. This is also true in response to injurious motion such as sudden inversion in the case of lateral ankle sprains. It is clear that while the peroneal muscles are the most commonly studied muscles in regards to sudden inversion, the tibialis anterior and triceps surae have consistently been shown to co-activate in an attempt to provide maximal joint stability (Delahunt, Monaghan et al. 2006; Ty Hopkins, McLoda et al. 2007). This is a very effective injury prevention strategy, however due to the fact that we chose to focus on peroneal fatigue, it is possible that despite fatigue of the peroneus longus, co-activation of other dynamic stabilizers at the ankle acted to maintain stability. Because the muscle activation of tibialis anterior and the triceps surae were not measured and because these muscles were not subjected to a focused fatigue protocol, we may be less able to truly understand the effect a more functional fatigue protocol may have had on ankle stiffness.

Limitations
The primary limitation in this study was the potential inefficiency in the fatigue protocol selected. While subjects were fatigued according to the operational definition provided in this paper, median power frequency data collected during testing sessions showed no significant change when comparing pre-fatigue and post-fatigue maximal volitional contractions. While there has been debate as to the validity of this measure and decreases of 10% of force output were seen in subjects, the inability to confirm this fatigue with a more quantitative measure that was independent of subject effort remains a limitation (Edwards 1981; Vollestad 1997; Krivickas, Taylor et al. 1998; Dimitrova and Dimitrov 2003).

The reliance on subject effort was also a limitation. Subjects were repeatedly instructed to provide maximal effort on all tasks and verbal cues were utilized throughout the testing session, however it remained difficult to gauge subject effort during both MVIC and sustained hold portions of the fatigue protocol despite the visual display with target. Subject effort was especially important at the end of the sustained hold protocol because it was the responsibility of the subject to maintain contraction until they were physically unable to produce force matching 10% of MVIC. While the visual display allowed us to monitor force output, it did not allow for a gauge of the effort required by the subject to maintain the fatiguing contraction and therefore forced us to rely on the motivation of the subject. This may have affected but the calculation of 20% MVIC and the duration for which the subject maintained the fatiguing contraction.

Subject positioning during both MVIC and sustained hold isometric contractions was also a limitation. While efforts were made in order to prevent compensatory motions, such as a strap around the shank, patients were in a seated position with the foot in the position of
most comfort during the testing session. This position may have led to contribution from other muscles in order to both increase force output and elongate the time to failure in the fatigue protocol. It is possible that the subject may have utilized both hip internal rotators and ankle dorsiflexors in an attempt to improve performance during the tasks required during testing despite confirmation of peroneus longus activation throughout the task via EMG activity.

Future Research

The effect of fatigue on ankle and ankle instability remains an important area that will require a great deal more research in order to understand both the physiological effects of fatigue at the ankle and how they translate to an increased risk of injury. Future research should focus on testing ankle stability in a position that more realistically models an injurious mechanism including plantarflexion. This may allow us to more clearly see the differences between the stable and functionally unstable ankle. Future research should also focus on finding a more specific fatigue protocol that may work to fatigue postural leg muscles in a manner that is modeled after a more functional fatigue. This would allow for greater clarity to be achieved in both ankle stiffness and functional performance measures due to both greater confidence in fatigue of leg musculature that is related to that which is experienced during athletic activity, and the improved ability to transition future results toward clinical interventions that may aid in preventing injury.

Conclusion
There are several factors that may have had an effect on the outcome of this study including testing position, insufficient fatigue of the peroneals, and a lack of more global fatigue of ankle musculature. However, when comparing our results with those of previous studies, it is becoming clear that there is no measurable difference in ankle stiffness between stable and functionally unstable ankles when tested in a neutral joint position both pre-fatigue and post-fatigue despite some alteration in peroneal EMG characteristics. While this finding may not be clinically relevant on its own, in concert with future research, it may allow for a clearer understanding of compensatory mechanisms utilized by athletes with functionally unstable ankles in an attempt to prevent re-injury, as well as the physiological changes that may occur following fatigue that pre-dispose athletes to both initial and re-injury.
Appendix A

University of North Carolina-Chapel Hill
Consent to Participate in a Research Study
Adult Subjects
Biomedical Form

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Title of Study: The effects of fatigue and functional ankle instability on ankle stiffness

Principal Investigator: Christopher Kuenze
UNC-Chapel Hill Department: Department of Exercise and Sports Science
UNC-Chapel Hill Phone number: 732-779-8136
Email Address: ckuenze@email.unc.edu
Faculty Advisor: Dr. Steven M. Zinder

Study Contact telephone number: cc-779-8136
Study Contact email: ckuenze@email.unc.edu

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What are some general things you should know about research studies?
You are being asked to take part in a research study. To join the study is voluntary.
You may refuse to join, or you may withdraw your consent to be in the study, for any reason.

Research studies are designed to obtain new knowledge that may help other people in the future. You may not receive any direct benefit from being in the research study. There also may be risks to being in research studies.

Deciding not to be in the study or leaving the study before it is done will not affect your relationship with the researcher, your health care provider, or the University of North Carolina-Chapel Hill. If you are a patient with an illness, you do not have to be in the research study in order to receive health care.

Details about this study are discussed below. It is important that you understand this information so that you can make an informed choice about being in this research study. You will be given a copy of this consent form. You should ask the researchers named above, or staff members who may assist them, any questions you have about this study at any time.

What is the purpose of this study?
The purpose of this research study is to learn about the effects of fatigue and functional ankle instability on ankle joint stiffness. Lateral ankle sprains are one of the most common injuries in sports, resulting in high costs of treatment and time lost from normal activity. Lateral ankle sprains accounted for 40% of all volleyball injuries, while it has been reported that 30% of all injuries in high school basketball involved the ankle joint. In addition, initial ankle sprains may predispose the ankle to chronic instability and degeneration. Therefore, the
prevention of an initial ankle sprain is essential. Joint stability is affected by several factors, including joint stiffness. A measurement of the stiffness of the ankle joint can be considered a measurement of ankle joint stability.

Fatigue of the muscles surrounding the ankle joint has been shown to affect the ability to control motion of the ankle joint in both healthy and functionally unstable ankles. A fatigued muscle has a decreased ability to produce force and may leave the joint more vulnerable to injury. However, it remains unclear if fatigue of these muscles during activity has an actual effect on the stability or stiffness of the ankle. Therefore, the evaluation of fatigue of the muscles surrounding the ankle will allow us to better understand the effects of fatigue associated with activity on the stability of the ankle in both healthy and functionally unstable ankles and may clarify strategies for preventing injury during activity.

**Are there any reasons you should not be in this study?**
You should not be in this study if you have
- History of lower extremity injury that required physician’s assessment within six months of testing
- Current symptoms of ankle sprain
- History of ankle fracture and/or current participation in formal physical therapy of the lower extremity
- History of concussion, balance or vestibular disorders
- Any medical condition that might affect or be compromised by participation in this study

**How many people will take part in this study?**
If you decide to be in this research study, you will be one of approximately 40 people enrolled as subjects.

**How long will your part in this study last?**
You will participate in one 60-minute testing session.

**What will happen if you take part in the study?**
- You will report to the Sports Medicine Research Laboratory for your testing session
- You will fill out a consent form and have time to ask any questions you may have upon entering the Research Lab.
- You will fill out a questionnaire about your ankle and will be assigned to a research group. The testing session will be exactly the same regardless of the group to which you are assigned. You may choose not to answer a question for any reason.
- Sensing electrodes will be placed over three of the muscles in your leg and will be held to the skin with items similar to Band-aids. In order to assure good contact, the areas of your leg under the electrodes may need to be shaved, lightly abraded, and cleaned with an alcohol wipe. These electrodes will remain on your leg throughout the testing session.
You will be asked to remove your shoes and sit with the foot being measured in the inversion/eversion swaying cradle (Figure 1). After you achieve a comfortable seated position, fifty percent of your body mass will be positioned over the involved knee to simulate standing on two feet.

The cradle will swing back and forth. The total movement of your ankle will be less than one inch in each direction, which is well within the normal limits of your ankle motion. You will complete a total of five trials at this time.

You will then move to a table where a tool called a load cell will be used to measure the fatigue of the muscles surrounding your ankle. You will sit down on the table with your leg stabilized by small straps.

You will then evert your foot (“move your foot out”) as hard as you can for 3 trials of 5 seconds each trial. The strength of your contraction will be monitored on a visual display and will be recorded for each trial.

After a brief rest, you will be asked to evert your foot (“move your foot out”) and hold a contraction that is a small percentage of your maximum contraction for as long as you can. Your contraction will be monitored on a visual display and you will be told to stop contracting once your muscles are fatigued to a pre-determined point.

You will quickly move from the load cell to the inversion/eversion swaying cradle (Figure 1).

You will be positioned in the same manner as the initial 5 trials and will undergo 5 additional trials of back and forth oscillation at this time.

Following the last of the 5 trials, your testing session will be over. You will have time to ask any questions that you may have.

What are the possible benefits from being in this study?
Research is designed to benefit society by gaining new knowledge. You will not benefit personally from being in this research study.

What are the possible risks or discomforts involved with being in this study?
The risks associated with participation in this study are minimal.

- There is a small risk of injury to the muscles surrounding the ankle during the fatigue protocol. However, due to the low amount of force (20% of maximum capacity) you will exert during the protocol and your physical activity level, this risk can be minimized. It is also possible that you may experience muscle soreness surrounding the ankle following the experiment due to this fatigue protocol.

- You may feel slight discomfort during ankle stability testing due to the addition of the 50% body mass load, but previous testing on the stiffness apparatus has caused no ill effects. There is also a small risk of ankle injury while on the testing cradle.

- You may experience minor skin abrasions and discomfort will be possible during and following skin preparation for EMG electrodes. There may be uncommon or previously unknown risks. You should report any problems to the researcher.

- In addition, there may be uncommon or previously unknown risks that might occur. You should report any problems to the researchers.

What if we learn about new findings or information during the study?

56
You will be given any new information gained during the course of the study that might affect your willingness to continue your participation.

**How will your privacy be protected?**
You will not be identified in any report or publication about this study. You will be assigned an identification number (ID) for data collection and all data will be stored on computers in the Sports Medicine Research Lab where a password is necessary for access. The only door to enter the lab is locked with key card access to ensure privacy. Only members performing research have access to these computers, therefore identification is very unlikely. Your consent forms will be destroyed 12 months after the submission of the final report of this study.

No subjects will be identified in any report or publication about this study. Although every effort will be made to keep research records private, there may be times when federal or state law requires the disclosure of such records, including personal information. This is very unlikely, but if disclosure is ever required, UNC-Chapel Hill will take steps allowable by law to protect the privacy of personal information. In some cases, your information in this research study could be reviewed by representatives of the University, research sponsors, or government agencies for purposes such as quality control or safety.

**What will happen if you are injured by this research?**
All research involves a chance that something bad might happen to you. This may include the risk of personal injury. In spite of all safety measures, you might develop a reaction or injury from being in this study. If such problems occur, the researchers will help you get medical care, but any costs for the medical care will be billed to you and/or your insurance company. The University of North Carolina at Chapel Hill has not set aside funds to pay you for any such reactions or injuries, or for the related medical care. However, by signing this form, you do not give up any of your legal rights.

**What if you want to stop before your part in the study is complete?**
You can withdraw from this study at any time, without penalty. The investigators also have the right to stop your participation at any time. This could be because you have had an unexpected reaction, or have failed to follow instructions, or because the entire study has been stopped.

**Will you receive anything for being in this study?**
You will not receive anything for taking part in this study.

**Will it cost you anything to be in this study?**
It will not cost you anything other than the potential cost of parking during on campus to participate in this study. If needed, parking passes can be made available for use during the testing session.

**What if you are a UNC student?**
You may choose not to be in the study or to stop being in the study before it is over at any time. This will not affect your class standing or grades at UNC-Chapel Hill. You will not be
offered or receive any special consideration if you take part in this research.

**What if you are a UNC employee?**
Taking part in this research is not a part of your University duties, and refusing will not affect your job. You will not be offered or receive any special job-related consideration if you take part in this research.

**What if you have questions about this study?**
You have the right to ask, and have answered, any questions you may have about this research. If you have questions, or if a research-related injury occurs, you should contact the researchers listed on the first page of this form.

**What if you have questions about your rights as a research subject?**
All research on human volunteers is reviewed by a committee that works to protect your rights and welfare. If you have questions or concerns about your rights as a research subject you may contact, anonymously if you wish, the Institutional Review Board at 919-966-3113 or by email to IRB_subjects@unc.edu.

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**Title of Study:** The effects of fatigue and functional ankle instability on ankle stiffness

**Principal Investigator:** Christopher Kuenze

**Subject’s Agreement:**
I have read the information provided above. I have asked all the questions I have at this time. I voluntarily agree to participate in this research study.

<table>
<thead>
<tr>
<th>Signature of Research Subject</th>
<th>Date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Printed Name of Research Subject</td>
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<table>
<thead>
<tr>
<th>Signature of Person Obtaining Consent</th>
<th>Date</th>
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<tr>
<td>Printed Name of Person Obtaining Consent</td>
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## Appendix B
### Subject Information Sheet

<table>
<thead>
<tr>
<th>Subject #________________</th>
<th>Date Tested________________</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age________________________</td>
<td>Sex________________________</td>
</tr>
<tr>
<td>Leg Dominance_______________</td>
<td>Stable/Unstable______________</td>
</tr>
<tr>
<td>Height (cm)_________________</td>
<td>Weight (kg)_________________</td>
</tr>
<tr>
<td>Ankle Tested_______________</td>
<td></td>
</tr>
</tbody>
</table>

**Ankle Instability Instrument**

1. Have you ever sprained an ankle?  
   - [ ] Yes  
   - [ ] No

2. Have you ever seen a doctor for an ankle sprain?  
   - [ ] Yes  
   - [ ] No  
   2a. How did the doctor categorize your most serious ankle sprain?  
      - [ ] Mild (grade 1)  
      - [ ] Moderate (grade 2)  
      - [ ] Severe (grade 3)

3. Did you ever use a device (such as crutches) because you could not bear weight due to an ankle sprain?  
   - [ ] Yes  
   - [ ] No  
   3a. In the most serious case, how long did you need to use the device?  
      - [ ] 1-3 Days  
      - [ ] 4-7 Days  
      - [ ] 1-2 Weeks  
      - [ ] 2-3 Weeks  
      - [ ] >3 Weeks

4. Have you ever experienced a sensation of your ankle "giving way"?  
   - [ ] Yes  
   - [ ] No  
   4a. When was the last time your ankle "gave way"?  
      - [ ] <=2 years ago

5. Does your ankle ever feel unstable while walking on a flat surface?  
   - [ ] Yes  
   - [ ] No

6. Does your ankle ever feel unstable while 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
References


